YALE

MEDICAL LIBRARY

The Role of CNS Dopamine Pathways in the mediation of Amphetamine Induced Stereotyped Behavior in Rats Behavioral and Histochemical Investigations and Clinical Relevance

Submitted by Irving M. Asher, March 1, 1974 to the Dept. of Psychiatry, Yale University School of Medicine, in partial fulfillment of the requirements for the degree of Doctor of Medicine

The Role of CNS Dopamine Pathways in the mediation of Amphetamine Induced Stereotyped Behavior in Rats

Behavioral and Histochemical Investigations and Clinical Relevance

...whatever be our activity, facts go quicker than we, and we cannot catch them; while the scientist discovers one fact, there happen milliards of milliards in a cubic millimeter of his body.

H. Poincaré

The palm at the end of the mind,
Beyond the last thought, rises
In the bronze decor,

A gold-feathered bird
Sings in the palm, without human meaning
Without human feeling, a foreign song.

You know then that it is not the reason
That makes us happy or unhappy.
The bird sings. Its feathers shine.

The palm stands on the edge of space.
The wind moves slowly in the branches.
The bird's fire-fangled feathers dangle down.

"Of Mere Being", W. Stevens

Table of Contents

Figures and Tables

Introduction

In recent years much evidence has accumulated to suggest that a pathophysiologic functioning of CNS dopaminergic neural systems may play an etiologic role in schizophrenia (Randrup and Munkvad, 1971; Klawans et al., 1972; Snyder, 1972 Stevens, 1973). This hypothesis has arisen in part from a fascinating reciprocity of certain neurologic and psychiatric disorders.

1. Parkinson's disease which presents clinically with the triad of resting tremor, rigidity and bradykinesia, is characterized pathologically most consistently by degeneration of the melanin containing nuclei of the brainstem, esp. the substantia nigra (Blackwood et al., 1963). The substantia nigra has been shown by histoflourescent techniques to contain dopaminergic cell bodies whose axons project rostrally to the ipsilateral striatum (Anden et al., 1964; Ungerstedt, 1971). The hypothesis that an alteration of dopamine metabolism due to the nigral degeneration is important in the pathogenesis of Parkinson's disease (Hornykiewicz, 1966) led to the attempt to treat this disorder by administering to patients the dopamine precursor L-Dopa (Cotzias et al., 1967). The success of this pharmacotherapy has led to large numbers of Parkinsonian patients on chronic large doses of L-Dopa. This population has been found to manifest a high percentage of psychiatric disorders, including fullblown psychosis directly attributable to the L-Dopa therapy (Celesia and Barr, 1970). Furthermore,

the development of psychosis in these patients is often associated with the concurrent development of dyskinetic movements, the most common of which is a bucco-lingual dyskinesia. Both the psychosis and the dyskinesia resolve with lowering of the L-Dopa dose.

2. Huntington's chorea, a dominantly inherited disorder characterized by dyskinesia, hyperkinesia, hypotonia and dementia is most often found to show on pathologic investigation a degeneration of caudate nucleus, putamen, and frontal cortex (Blackwood et al., 1963). This disease has been noted to be associated with psychosis since the time it was first described (Huntington, 1872). Hallucinations and paranoid behavior are common in patients with this disease (Bolt, 1970) and schizophrenia has been considered the most common concommitant disorder (Brothers, 1964). While organic dementia ultimately develops, the earliest presentation of this disease may be a psychiatric disorder not infrequently misdiagnosed as schizophrenia. It has been suggested that the motor disturbance results from an increased sensitivity of dopamine receptors in the striatum (Klawans et al., 1972). This hypothesis has led to the successful use of L-Dopa as a provocative test for young individuals genetically at risk (Klawans et al., 1972b). The motor disturbance has been shown to respond to treatment with neuroleptic, dopamine blocking agents (Fog and Pakkenberg, 1970). It has recently been reported that the GABA is reduced in the basal ganglia of patients with Huntington's chorea (Perry, et al., 1973).

Though the significance of this finding is uncertain, there is evidence that GABA may play a role as an inhibitory neurotransmitter. It is further worth noting that psychiatric disorders are very commonly associated with other choreatic disorders as well (Freeman et al., 1965).

3. Antipsychotic drugs of the phenothiazine class and the butyrophenone haloperidol increase dopamine turnover in the striatum (Nyback and Sedvall, 1968; O'Keefe et al., 1970), apparantly as a result of dopamine receptor blockade and a compensatory neuronal feedback (Carlsson and Lindquist, 1963; Bunney et al., 1973). Since their therapeutic introduction by Delay and Deniker (1952) these drugs have led to a revolution in the management of psychosis. The widespread use of these agents has however also been responsible for the appearance of several new neurologic disorders. A drug induced Parkinsonism is relatively common among patients on chronic antipsychotic medications. It should be noted that attempts to treat this drug-induced Parkinsonism by the administration of L-Dopa had to be curtailed due to the exacerbation of the psychosis (Yaryura-Tobias, 1970).

Another group of disorders linked to chronic antipsychotic use is the tardive dyskinesias (Crane, 1968; American College of Neuropsychopharmacology, 1973), which often present with a bucco-lingual dyskinesia. This disorder frequently becomes manifest after lowering or discontinuing antipsychotic medications (though it does occur without alterations of dosage) after

prolonged periods of use. It has been suggested that the tardive dyskinesias result from a hypersensitivity of dopamine receptors caused by a chemical denervation by prolonged exposure to blocking agents (Klawans et al., 1972).

4. Amphetamine has been shown to be capable in high doses of producing in humans a psychosis often indistinguishable from an acute paranoid schizophrenia. Snyder (1972) has recently offered a review of the pertinent literature. Amphetamine has been shown to have several actions on central monoamines, the most important of which appears to be the release of newly synthesized catecholamines from presynaptic nerve endings (Anden, 1970; Carlsson, 1970; Fuxe and Ungerstedt, 1970). It is of note that the psychosis induced by amphetamine is often accompanied by stereotyped activities and responds well to treatment with antipsychotic phenothiazines.

The data presented above indicates that drugs which increase functionally active dopamine (DA) in the CNS (such as L-Dopa and amphetamine), while useful in the treatment of hypokinetic, rigid states (Parkinson's disease), tend to produce as side effects hyperkinesia, dyskinesia, hypotonia and psychosis. On the other hand, those drugs which decrease functionally active DA (phenothiazines and haloperidol) are useful in the treatment of hyperkinetic, dyskinetic and psychotic states, but tend to produce Parkinsonian side effects. This apparant wedding of psychic and motor responses to changes in central dopaminergic activity has led Klawans (Klawans et al.,

1972) to suggest a role for striatal dopamine in the pathogenesis of schizophrenia.

It is known however from histoflourescent microscopic investigations that there are 3 dopaminergic pathways in the CNS (Ungerstedt, 1971). (See figures 1 & 2) 1) With cell bodies in the substantia nigra zona compacta (cell group A9, as named by Dahlstrom and Fuxe (1965)) projects to ipsilateral caudate and putamen - the nigrostriatal, NS, pathway; 2) With cell bodies lying just dorsal to the nuc. interpeduncularis (A10) projects rostrally to distribute terminals to the olfactory tubercle (OT), nuc. accumbens, nuc. interstitialis stria terminalis (dorsalis), and the nuc. amygdaloideus centralis. This projection has been termed the meso-limbic, ML, pathway; 3) Cell bodies are located within the nuc. arcuatus (A12) and project to innervate the median emminence- the tuberoinfundibular system. (Two more groups of DA containing cells have been described but their projections have not as yet been determined. The A8 group lies just caudal to the substantia nigra and probably projects to striatum. The A13 group lies dorsolateral to the nuc. dorsomedialis hypothalami and may give rise to a rostral projection joining the medial forebrain bundle , MFB.

The first of the DA projections has been convincingly invoked in the pathophysiology of Parkinson's disease. The third, tubero-infundibular system, has been implicated in the

Fig 1

Anatomy of CNS DA Pathways in the Rat

(after Ungerstedt, 1970)

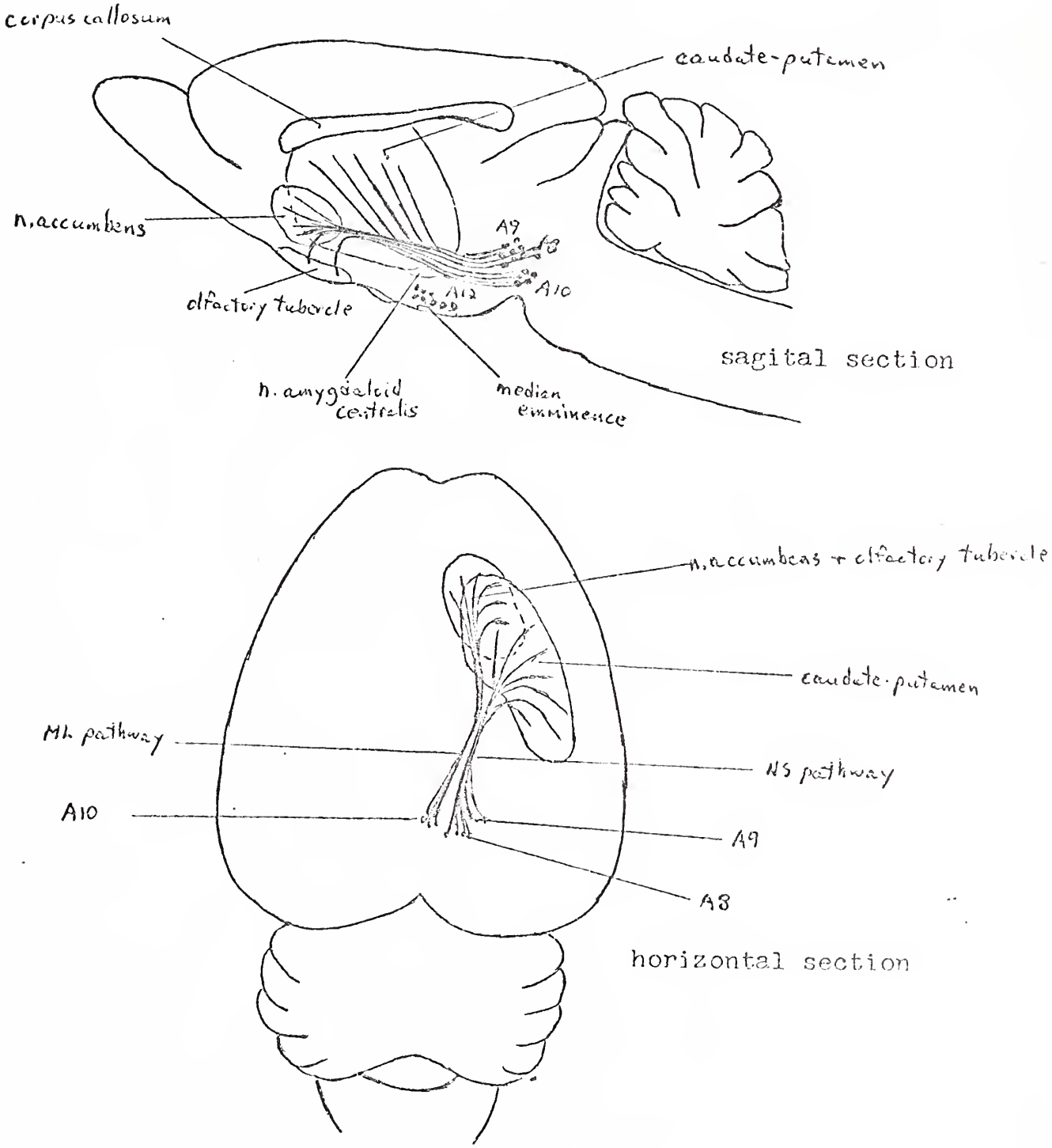
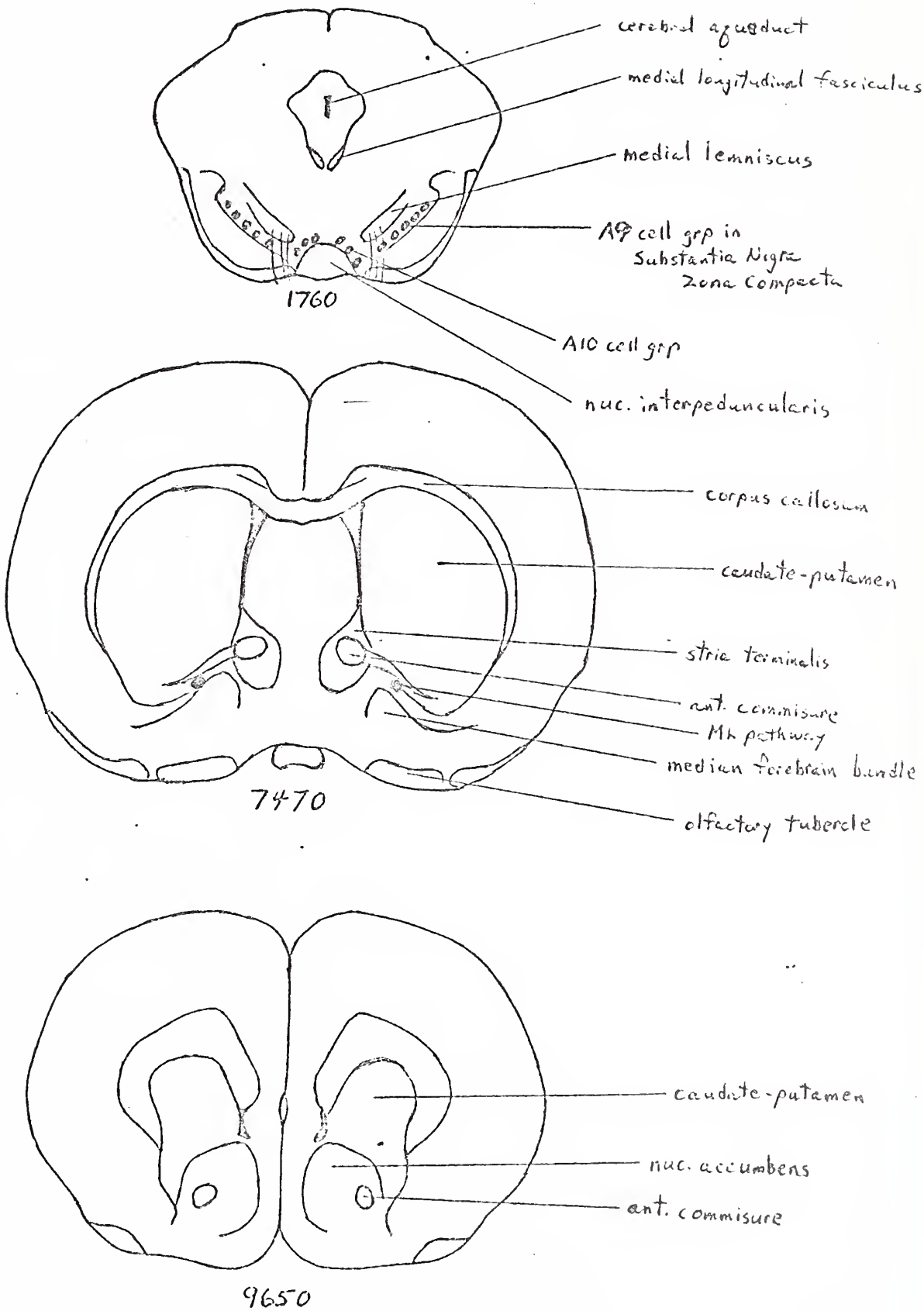


Fig 2.

Anatomy of CNS DA Pathways

Frontal Sections



regulation of gonadotropin secretion from the anterior pituitary (Fuxe and Hokfelt, 1969; Kamberi et al., 1970). The meso-limbic pathway remains obscure however with regard to its function.

Wilson (1972) has suggested that the olfactory tubercle, OT, and nuc. accumbens, NAc, form a unitary structure, traversed by the MFB, that may be thought of as a "ventral striatum". The OT and NAc receive afferents from the hippocampus, piriform cortex, and ventral tegmentum (A10) and send efferents to the substantia innominata and DM nuc. of the thalamus. Their anatomical links thus make the OT and NAc prominent members of the forebrain limbic system. On the basis of marked identities in histomorphologic appearance it has been suggested that the "ventral striatum" and its projection to substantia innominata are the limbic equivalent of caudate-putamen and its projection to globus pallidus. The parallel between OT-NAc and caudate-putamen is futher enhanced by the projection to each of a specific DA pathway from the midbrain. The limbic nature of OT-NAc may be further emphasized by its afferent connection to the A10 group which lies in the ventral tegmental area of Tsai, part of Nauta's "midbrain limbic area" (Nauta, 1958).

The existence of two distinct ascending DA pathways allows for some modification of the unitary DA-motor-psychosis hypothesis elaborated by Klawans. While striatal DA may be involved in the pathogenesis of psychosis, none of the data presented would be

inconsistent with the parcelling of motor phenomena to the NS DA system and psychic phenomena to the ML system. This alternative is attractive from the standpoint of the neural associations of the two systems.

The very great incidence of a schizophrenic-like psychosis in patients with temporal lobe epilepsy (Slater et al., 1963; Falconer, 1973) some of whose major characteristics are the production of behavioral automatisms, lip-smacking and chewing motions and olfactory hallucinations make the ML system seem a reasonable substrate for some of the motor phenomena (automatisms and buccolingual dyskinesias) as well as for the development of psychosis. Furthermore, the ability to alter autonomic state and elicit sniffing, licking and tonic and clonic movements with stimulation of the amygdala adds to the data suggesting a possible motor role for the ML system.

Amphetamine in large doses produces in rats a stereotyped behavior that has long been used as an animal model of psychosis (Janssen et al., 1961; Randrup and Munkvad, 1970). In an attempt to determine the possible roles of the NS and ML systems in psychosis it was attractive to attempt to determine their roles in the mediation of amphetamine induced stereotyped behavior (AISB).

Amphetamine Induced Stereotyped Behavior (AISB) and Amphetamine Induced Psychosis

Amphetamine in moderate doses is capable of eliciting nondistractable, driven, rapid and repetitious forms of behavior in many species (Randrup and Munkvad, 1967; Snyder, 1972). Snyder emphasizes that the common properties of these repetative motor sequences are their compulsiveness and their apparant purposelessness. These highly repetitive motor activities elicited by amphetamine appear to be species specific in lower animals- mice, rats, guinea pigs and cats - but become more individualized in primates.

In rats administered 10 mg/kg i.p. of d-amphetamine. stereotyped activity becomes maximal about 50 min. after injection and continues for about 2 hrs. (Fog, 1969). This stereotyped behavior is classically described as sniffing, licking and gnawing of the cage floor. Prior to the onset of stereotypy the rat shows the well known locomotor stimulant effect of amphetamine, but as stereotypy begins locomotion ceases (Randrup and Munkvad, 1970).

When administered amphetamine, pidgeons peck; cats exhibit sniffing and constant side to side looking movements and chimpanzees exhibit individualized repetitive activities such as side to side looking, self-picking and various more complex motor sequences.

Stereotyped compulsive behavior has also been reported as a concomitant of amphetamine psychosis in man (Scher, 1966; Rylander, 1966; Kramer et al., 1967). These activities may be of a highly individualized nature such as sorting out of a handbang's contents over and over again, or taking apart and reassembling watches or motors. Various bucco-lingual dyskinesias have also been described as associated with amphetamine psychosis (Ashcroft et al., 1965; Connell, 1966; Mattson and Calverly, 1968). Both auditory and visual hallucinations have been reported in amphetamine psychosis (Ellinwood, 1967), but as Snyder points out (Snyder 1972) the reports of visual hallucinations are more common in those cases where psychosis was precipitated by a single large dose of amphetamine than by long term low dose abuse, just as they are more characteristically seen in acute schizophrenic decompensations than in chronic psychosis without drugs (Chapman, 1966: Bowers and Freedman, 1966). Olfactory hallucinations are also characteristic of amphetamine psychosis, reported by 70% of the former amphetamine addicts that had experienced psychotic episodes interviewed by Ellinwood (1967). Finally, tactile hallucinations are also very common with amphetamine psychosis and they are frequently accompanied by delusions of parasitosis and compulsive rubbing, picking and scratching of the skin not unlike that of the stereotypies seen with lower animals.

Amphetamine has precipitated psychoses in volunteers after

one day of frequent oral doses (Griffith, 1972) and within one hour after large i.v. dosage (Bell, 1973). The psychoses would thus seem to be a direct effect of the drug and not the result of sleep deprivation or concurrent drug abuse of psychedelics or barbiturates. As has been mentioned, the psychosis responds to phenothiazines. It is also worth noting that the onset of the psychosis, which is invariably of a paranoid nature, is rather abrupt and is immediately preceded by a cessation of motor activities and a period of withdrawal not unlike the cessation of locomotor stimulation with the onset of AISB in rats (Ellinwood, 1967; Griffith, 1972).

Evidence for the mediation of AISB by CNS Dopamine

The depletion of central catecholamines by the administration of α-methyl-p-tyrosine, (AMPT), a tyrosine hydroxylase inhibitor, prevents the induction of both locomotor stimulation, LS, and stereotyped behavior by the subsequent administration of amphetamine to rats (Randrup and Munkvad, 1966; Weissman et al., 1966). Administration of L-Dopa and an MAO inhibitor produces a stereotyped behavior like that seen with amphetamine. This effect is not altered by DDC, a depletor of NE by DA-β-hydroxylase inhibition, but is prevented by AMPT (Scheel-Kruger and Randrup, 1967). FLA-63, another DA-β-hydroxylase inhibitor, is capable of decreasing hypermotility induced by

d-amphetamine in mice (Svensson, 1971) but does not alter AISB in rats (Ayhan and Randrup, 1972).

It would thus seem that the LS effect of d-amphetamine requires the presence of functional pools of NE, but that AISB requires only DA. Consistent with this is the finding that imipramine, which blocks reuptake by NE terminals but not by DA terminals, potentiates the LS response of high dose L-Dopa but not the induced stereotypy. (Friedman and Gershon, 1972).

Another approach to determining which of the CNS catecholamine systems mediates AISB has been the attempt to correlate behavioral differences of the d and l isomers of amphetamine with their specificities for NE and DA systems.

Coyle and Snyder (1969) reported that d-amphetamine is 10 times as potent as the l isomer in inhibiting reuptake of catecholamines into synaptosomes from nonstriatal brain tissue, while on striatal synaptosomes the two isomers are equipotent in blocking uptake. These results were confirmed (Taylor and Snyder, 1970) in studies of the uptake of H^3 - NE into brain slices after amphetamine pretreatment of the rats. Again, d and l were equipotent in blocking uptake into striatum. l was ineffective in other brain regions however, whereas d showed significant block of uptake into brainstem, cerebellum and diencephalon. D was also effective in depleting endogenous NE stores whereas l was not. In behavioral studies rats were pretreated with the MAO inhibitor iproniazid and the ED_{50}

for LS and AISB determined for the two isomers. The endpoint for determination of AISB was chewing, licking and gnawing resumed within 10 sec. after disturbance. They reported

ED_{50} mg/kg

	LS	AISB
d	0.9	2.1
l	8.8	4.4

again confirming a 10 times greater potency of d-amphetamine on locomotor stimulation - thus indicating a primary NE mediation, but only a 2:1 difference of their potencies for AISB - which it was concluded demonstrated a predominant role of DA in mediating AISB, with perhaps some minor NE effect.

Further evidence for a primary role of DA in the mediation of AISB comes from the observation that apomorphine, a direct DA receptor agonist (Anden et al., 1967),can also produce a stereotyped compulsive gnawing syndrome in rats (Harnack, 1874; Ernst, 1966). Apomorphine has been shown to mimic amphetamine in its ability to decrease the firing rate of dopaminergic neurons, but does not alter the firing of NE cells in the locus coeruleus (Bunney, 1973). The cessation of firing is caused by apomorphine even after the administration of AMPT which prevents the effect of amphetamine on these cells.

While sedative hypnotics have no efficacy whatever in blocking AISB, the antipsychotic neuroleptics do. In fact, the ability to block AISB has been used as a screening test for possible new antipsychotic agents (Janssen, 1961). Promazine,

a phenothiazine devoid of antipsychotic activity does not block AISB (Del Rio and Fuentes, 1969) and in general the antipsychotic potency of a drug seems to parallel its ability to block AISB. Clozapine, a potent new antipsychotic, however, has been reported to display only a very weak antagonism of AISB (Stille and Hippius, 1971). It is of particular note also that this drug has been reported to be free of extrapyramidal side effects. Furthermore, Anden (1973) has reported that clozapine effects limbic DA metabolism to a greater extent than it does striatal DA metabolism, whereas haloperiodol, an antipsychotic with prominent extrapyramidal side effects is equipotent on limbic and striatal DA metabolism. Such differences might be attributable to a differential blockade of limbic and striatal DA receptors. It thus becomes possible that the antipsychotic and extrapyramidal effects of the neuroleptics may be separable, and that AISB may be a more accurate model for extrapyramidal effects than psychosis.

Further evidence to suggest that AISB may be a better model for extrapyramidal motor effects than psychosis comes from looking at the effect on AISB of alterations in CNS acetylcholine. It is standard practice in this country to use anticholinergics for the alleviation of the extrapyramidal symptoms induced in schizophrenics by their neuroleptic medications. Kline and Davis (1969) go so far as to recommend the prophylactic use of anticholinergics in patients receiving antipsychotic medications, and it is the general consensus that such treatment does not alter the antipsychotic potency of the

medications. Even a report to the contrary (Singh and Smith, 1973) found that while it is possible that administration of benztropine may have reversed the effect of antipsychotics in promoting ease of social functioning, it did not appear to have any significant effect on either thought disorder or hallucinations. Thus it seems possible to ameliorate the motor effects of the neuroleptics by the use of anticholinergics without altering their antipsychotic effect. Anticholinergics have been shown to decrease, and anticholinesterase inhibitors to increase, the threshold dose of d-amphetamine required to induce stereotyped behavior. (Fog et al., 1966; Janowski et al., 1972; Klawans et al., 1972). Thus, with respect to its response to manipulations of cholinergic tone AISB appears more closely correlated with the motor effects of the neuroleptics than it does with the antipsychotic effects.

Perhaps then if the antipsychotic and extrapyramidal effects of the neuroleptics are pharmacologically separable they may also be anatomically separable and reflect the differential activities of these agents on the two ascending dopamine pathways.

Anatomic Substrate of AISB

Attempts to determine which of the two DA pathways mediates AISB have been inconclusive. Those studies in which DA, amphetamine, apomorphine or other drugs have been stereotaxically implanted into the striatum (Ernst and Smelnik, 1966; Fog et al., 1967;

Fuxe and Ungerstedt, 1970) while they have all concluded that striatal DA mediates stereotypy may be criticized on 2 counts. 1) It is difficult to control for diffusion of the substance from the head of the caudate to the nuc. accumbens which is rostro-ventrally contiguous with it. 2) The high concentrations of the drugs thus presented are most unphysiologic.

Lesions of the corpus striatum have been reported to reduce or abolish the stereotypies induced by amphetamine and apomorphine (Amsler, 1923; Fuxe and Ungerstedt, 1970; Anden, 1970; Naylor and Olley, 1972). Since there is evidence that stereotyped behavior is mediated by CNS DA and the striatum contains the largest concentration of DA in the brain, it has been concluded that the above lesions produce their effect by destroying striatal dopamine receptors. Aside from the lack of substantial histological proof of the specificity of these lesions (to rule out involvement of NAc or the ML pathway) a second objection to the above conclusion may be made. Gross electrolytic lesions destroy more than just the DA containing cells or their receptors. It is not impossible that the caudate, whose importance in the control of movement is well known, may be secondarily involved in the patterning of movements whose inception may be due to DA stimulation in the limbic areas. If this were the case then lesions of the striatum might block stereotypy not as a result of loss of striatal DA but by the alteration of the efferent processing of limbic DA activity.

Simpson and Iversen (1971) electrolytically lesioned the substantia nigra bilaterally and did not observe a diminishment of AISB. In a subsequent experiment however, (Creese and Iversen, 1972) when 6-hydroxydopamine (6-OHDA), which specifically destroys catecholamine containing cells while sparing other neural tissue (Ungerstedt, 1968; Bloom et al., 1969) was stereotaxically injected bilaterally, intra-cerebrally into the area just medial to the medial lemniscus in the midbrain, stereotypy to amphetamine was abolished. While Iversen concluded from this that it was the loss of striatal dopamine that prevented the occurence of AISB, it is obvious from the position of her cannula tip at injection that she must have lesioned both the NS and ML pathways since the two run together as they ascend through this area.

Finally, it was reported by McKenzie (1972) that removal of the olfactory tubercle bilaterally, but not of the striatum, resulted in the diminishment or absence of stereotypy to apomorphine in rats.

Thus, despite the voluminous published work on the subject, it is still uncertain 1) whether AISB is in fact a valid animal model of psychosis, and 2) whether AISB is mediated by the NS or ML DA pathways.

It thus seemed worthwhile to investigate further the roles of the NS and ML pathways in the mediation of AISB. Preliminary experiments were performed to delineate more precisely the behavior described as stereotypy and its response to some pharma-

cologic manipulations aimed at delimiting the respective roles of DA and NE in this behavior. After this, experiments were performed in which lesions were made of DA terminals in the forebrain by the intracerebral injection of 6-OHDA into either the corpus striatum or olfactory tubercle, and the subsequent response of the animal to amphetamine observed. While previous investigations have at most measured whole brain DA content as proof of the efficacy of lesion this has not allowed for confirmation of the specificity of the lesion. In these experiments advantage was therefore taken of the technique of histflourescent microscopy to specify the boundaries of DA depleted areas.

Methods

Male, albino Sprague Dawley rats weighing 230-270 grams were used in all experiments. Rats were fed a diet of Purina rat chow ad lib. Those rats who were lesioned in the caudate were tube fed with a 1:1 solution of sweetened condensed milk and water for one week after operation. For drug studies rats were used once and sacrificed.

The following drugs were used in the experiments:

d-amphetamine sulfate (varying doses as specified), routine behavioral testing with 10mg/kg

l-amphetamine sulfate (varying doses as specified)

pargyline hydrochloride (100 mg/kg)

apomorphine hydrochloride (varying doses as specified, routine behavioral testing with 5 mg/kg)

phenoxybenzamine (20 mg/kg)

clonidine hydrochloride (3 mg/kg)

All drugs were dissolved in distilled water and injected i.p.

Behavioral testing was carried out as discussed in the section on Stereotyped Behavior-Scoring System.

Animals to be lesioned were anaesthetized with chloral hydrate and mounted in a Kopf stereotaxic. 6-hydroxydopamine dihydrobromide was dissolved in distilled water with ascorbic acid .1 mg/ml to give a concentration of 5ug (free base)/ul.

The solution was injected through a 25ul Hamilton syringe at a rate of lul/min. The solution was always used within 3 days of preparation and never after any color change.

Preliminary experiments led to the establishment of coordinates for lesions as follows:

for lesions of the olfactory tubercle, skull landmarks were - anterior, bregma + 0.9mm.; lateral, 1.6mm.; vertical, 8.8mm. (from skull surface). These coordinates were found to correspond to a placement according to the atlas of Konig and Klippel (1963) of approx. A 7470u, L 2mm. and V -2.7mm.

lesions were also placed in the head of the caudate both centrally and dorsolaterally with coordinates according to Konig and Klippel of approximately A 8380, L. 2.4mm, V + 0.4mm and A 8380, L 3.5mm, V + 1.0mm respectively.

A few lesions were also made in the region of the ML bundle which was found to correspond to a position of A 7470, L 2.1, V-1.4.

All lesions were bilateral with 40ug of 6-OHDA injected into each side. After lesioning the animals were kept for two weeks to allow for adequate degeneration before behavioral testing was performed.

After behavioral testing the lesioned animals were decapitated, and the brains quickly removed. Cuts were made through the brain with a razor in the frontal plane at the most rostral

and caudal extents of the olfactory tubercle visible on the ventral surface. This tissue was then removed and quickly frozen in liquid propane-propylene in preparation for histoflourescence microscopy according to a modification of the formaldehyde condensation technique of Falk as previously described (Aghajanian and Asher, 1971) .

Estimates of the extent of DA depletion from the caudates were made as follows:

The volume of the head of the caudate from its most rostral extent to the frontal plane of 7190 (the caudal limit of brain examined) was calculated from the atlas of Konig and Klippel. This came to approx. 18.3 mm^3 (unilateral). The degree of loss of flourescence was taken as a measure of the degree of loss of DA from the structure since DA accounts for essentially all the flourescence of the caudate.

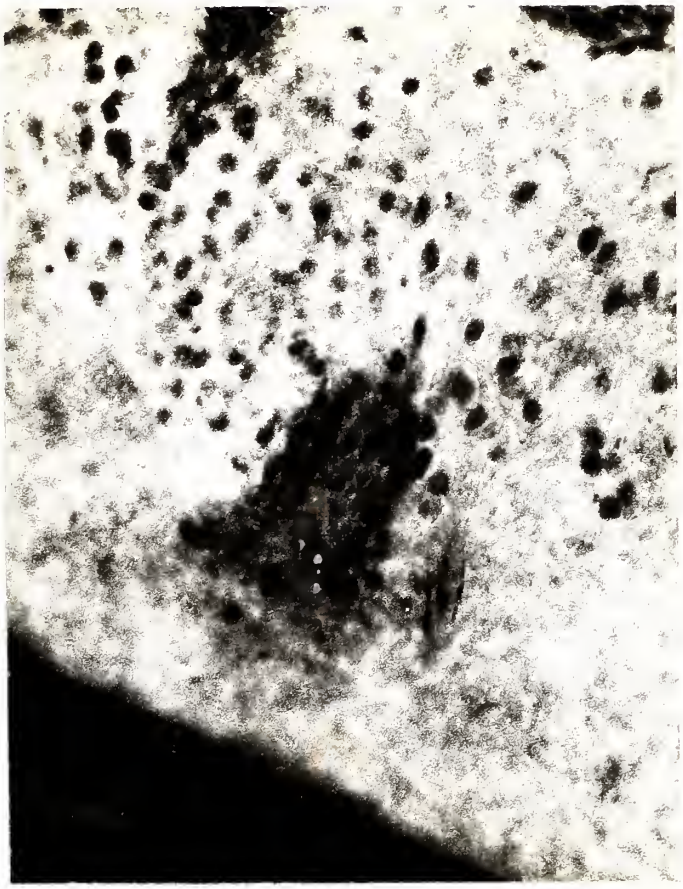
The caudate and OT-NAc in nonlesioned animals are normally marked by quite intense green flourescence of their native dopamine. (See figures 3 & 4). In lesioned animals however, this flourescence was markedly depleted. (See figures 3 & 4).

The extent of flourescence depletion was judged subjectively. Remaining areas of nondepleted caudate or tubercle were used as internal standards of 100% intensity of flourescence. Cortex was examined in each slide for determination of the background flourescence, rated 0%. Partially depleted areas of caudate or OT-NAc were rated as 25%, 50% or 75% intensity of flourescence

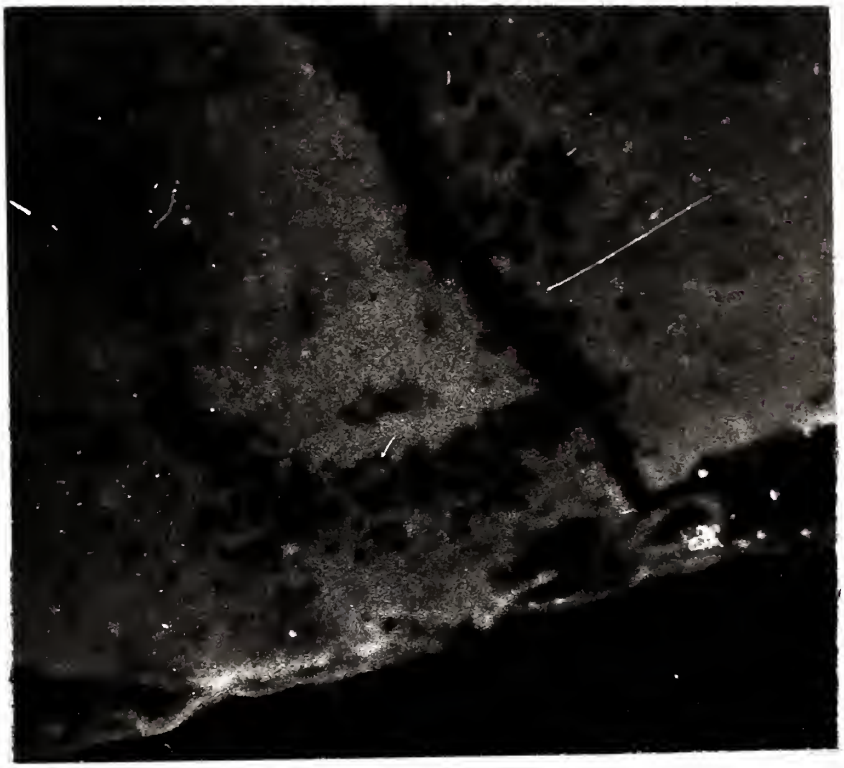
as compared to the internal standards of 100% and 0%.

The regions of absent or partial flourescence of caudate in slides at several rostro-caudal levels were mapped out and used to calculate a depleted volume. The percentage intactness of flourescence, as compared to a nonlesioned animal, was then calculated by dividing the nondepleted volume by the estimated real volume of 18.3. This number thus represented an approximation of the percentage flourescence, and presumably percentage of DA, of caput caudati in the lesioned animal as compared to that of a nonlesioned animal. A similar method was used for estimation of flourescent depletion of olfactory tubercle and nuc. accumbens.

Fig 3 (Legend see pg. 26)



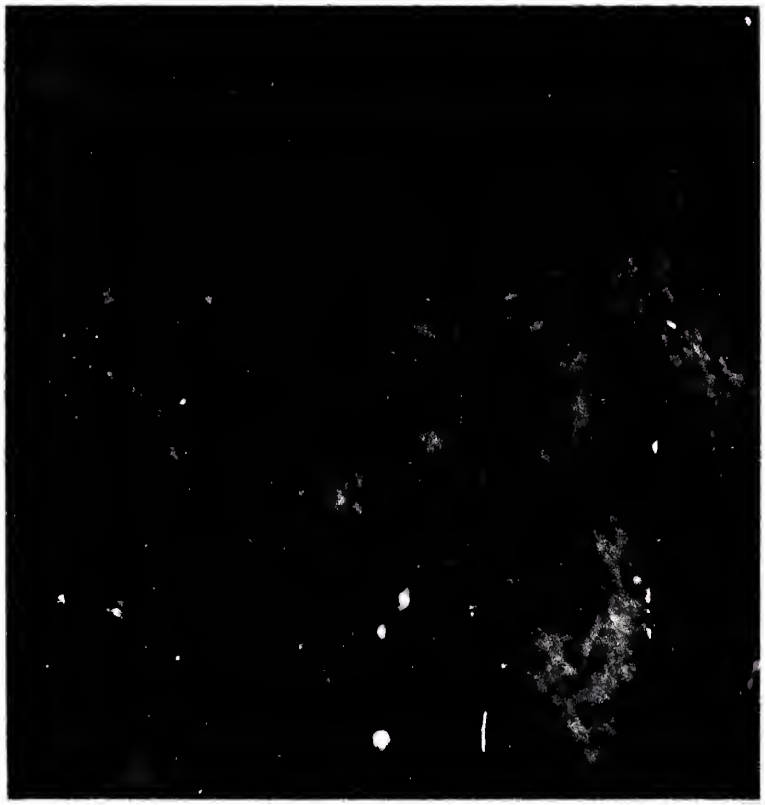
A



B

Fig 4 (Legend see pg. 26)

A



B

Legends to Figs 3 and 4

Comparison of Histoflourescence in control and 6-OHDA lesioned animals

Fig. 3) Histoflourescent micrographs of olfactory tubercle (100X)

A) bright flourescence of nonlesioned animal

B) absence of flourescence in animal lesioned with 6-OHDA in the olfactory tubercle

Fig. 4) Histoflourescent micrograph of caudate nucleus (60X)

A) bright flourescence of nonlesioned animal

B) marked depletion of flourescence in animal lesioned with 6-OHDA in the caudate nuc.

Stereotyped Behavior - Scoring System

Probably the most difficult problem encountered in the investigation of stereotyped behavior is that of defining and quantitating the phenomena observed. Snyder has noted stereotypy to be a "nondistractable, driven, rapid, and repetitious" sequence of motor activities. On a gross level such behavior is strikingly apparant to even the most unsophisticated observer. But to date no uniform criteria have been established for the scoring of this behavior in rats, and in the various investigations of AISB a vast multiplicity of scoring schemes has been used.

Harnack (1874) was the first to describe the stereotypy evoked in rats by apomorphine. Ernst has used the apomorphine induced "compulsive gnawing syndrome" as the primary focus of his investigations. Randrup and Munkvad on the other hand have been investigating the nature chiefly of amphetamine induced stereotypies. Though it has been pointed out that there is a qualitative difference in the stereotypies evoked by the drugs (Fog, 1969) the compulsive gnawing so characteristic of apomorphine has also frequently been used as an endpoint for tests of AISB. Since the mechanism of action of these two drugs is different and since amphetamine would stimulate NE and perhaps 5-HT systems as well as DA, while apomorphine would theoretically produce its effect on the DA system alone, it would not be unexpected for the behavior elicited by the two drugs to differ.

Randrup and Munkvad (1970) have succinctly described

the amphetamine induced behavior -

> The behavior we describe as stereotypy is seen very clearly in rats during a period about 1 to 2 hrs. after subcutaneous injection of 10 mg/kg d-amphetamine sulfate. The behavior consists of continuous sniffing, licking or biting of the cage floor or the animals own forelegs. The rat sits in a crouched posture and usually presses its body against the cage wall. Normal activities such as grooming, eating, rearing and forward locomotion are absent; backward locomotion is occasionally seen. This behavior is preceded and followed by periods of less complete stereotypy (sniffing is increased but interrupted by some other activities), which is also observed after small doses of amphetamine.

Lal and Soukes (1973) have described the apomorphine stereotypy to 5 mg/kg.

> Almost immediately after the injection of apomorphine continuous sniffing, licking and, in cages with sufficiently large grid size, biting developed. Initially some of the sniffing was directed upwards, with the animal rearing on its hind legs, but within 5 min. this was almost entirely directed towards the floor. Initially there was an increase in forward locomotion accompanying the compulsive behavior but within 10 min. normal activities such as grooming, social interaction and forward locomotion rarely occured. By this time ptosis was fully developed. In the terminal 10 min. there was a gradual return of normal activities interspersing the SB and diminution of ptosis until the animals grouped together and fell asleep or rested quietly.

One of the major differences in the descriptions above is that of the time of onset of stereotypy. There is also the ptosis noted with apomorphine, while amphetamine is usually noted to produce proptosis and wide palpebral fissures. While the two behaviors are similar they are also different. Is this due to a difference in relative drug potency on DA systems or is this due to a difference in monoamine involvement by the two drugs?

Janssen (1961) in testing the efficacy of neuroleptics to block AISB used compulsive gnawing as his endpoint, as have many others including Snyder and Coyle (1969) in their comparison of the d and l isomers. Attempts to quantitate intermediate grades of stereotypy have led to several different scoring systems (del Rio and Fuentes (1969); Ernst (1966); McKenzie, 1972; Naylor and Olley, 1972) in which the degree of drivenness, continuity and nondistractability of gnawing and licking behavior has been the major parameter quantitated. Other schemes (Naylor and Olley, 1972) while similar also take into account the appearance of "small head movements". Creese and Iversen (1972) quantitated the number of these small head movements /min. as their sole measure of AISB. There have thus been differences in the behaviors measured by various investigators. It becomes critical for the investigator to specify precisely what in fact he is measuring.

Animals in this experiment were observed for stereotypy by placing them in wire mesh cages 36 x 20 cm, two animals to a cage. They were allowed to acclimate to the cage for 20 min. after which drugs were administered. Each animal was observed for about 5 min. before rating its behavior at any given time point. The highest level of stereotypy shown by the animal during that time was given as the animal's score at that time point, unless the animal very clearly alternated rather equally between two levels during the 5 min. of observation, in which case both grades were scored and an intermediate grade used for

calculations. All animals were scored by the investigator. Animals were generally scored at 30, 60, 90 and 120 min. after injection of amphetamine and at 5 to 15 min. after apomorphine. On the basis of observations of many animals at several dose levels the following scoring system was arrived at and used in all experiments.

Stereotyped Behavior

Grade 0) Animal rests quietly, occasionally performing brief exploratory activities with discontinuous sniffing.

Grade EA) Animal is continually absorbed with exploratory activity - constantly ambulating around his cage and sniffing.

(Scored numerically as grade 0)

Grade 1) Animal sniffs the air with head raised, often moving his head in a circular manner such that his nose describes a circle in the frontal plane. His feet are fixed in position and he is immobile while performing this behavior, - but this behavior is often interspersed with mobile exploratory activity.

Grade 2) Animal bends his head so that his nose is now down to the cage floor. He moves his head from side to side, all the while sniffing. This activity may be performed with either wide or narrow lateral excursions of the head, may be

quite rapid or slow, and may or may not involve the poking of the animal's nose through the floor.

Grade 3) Animal continues behavior as in 2), but now begins to intermittently lick and or gnaw at the bottom of the cage.

Grade 4) Animal continuously gnaws or licks the cage bottom with total absence of ambulation.

The degree of compulsivity, measured by the inverse of the time it took the animal to return to his former behavior after being distracted, generally increased with increasing grade score. This scoring scheme was found to generally reflect the temporal onset sequence of behaviors after a dose of amphetamine as well as the gradation of responses to different dosage levels.

Two or three control animals were run in all experiments. Due to the very minor variation from day to day in response of control animals to 10 mg/kg of d-amphetamine or 5 mg/kg d-amphetamine plus pargyline, these controls from all experiments were grouped for final scoring.

Drug Studies

Some preliminary experiments were run in order to determine an optimum dose of d-amphetamine for behavioral testing and also to gain some insight into the relative roles of the various monoamines in the mediation of AISB.

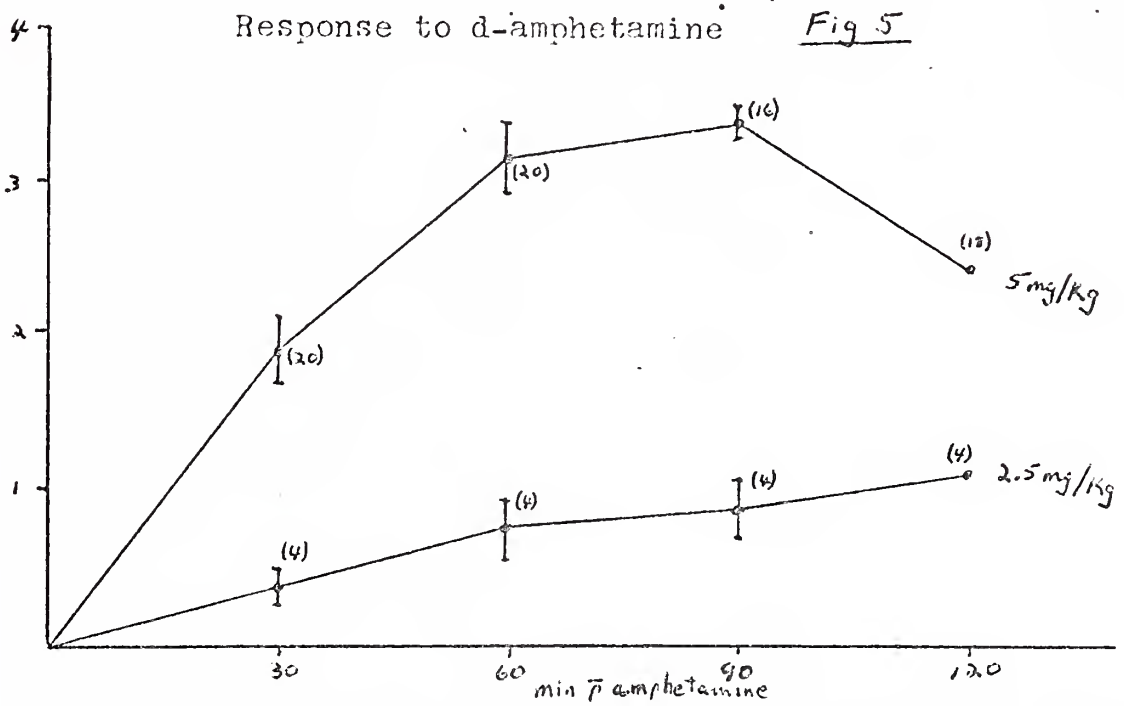
1) d and l amphetamine with MAO inhibition

Many reserachers have used MAO inhibitors to strengthen the observed stereotypy to amphetamine. In this first series of tests varying doses of d (2.5 and 5 mg/kg) and l-amphetamine (5, 7 1/2, 10 and 15 mg/kg) were administered to animals two hrs. after the prior injection of pargyline hydrochloride (100 mg/kg). Animals were scored at 30, 60, 90, and 120 min. after the injection of amphetamine. The individual scores of the animals at each time period were summed and divided by the number of animals to arrive at an average group score. Each animal was used once and sacrificed.

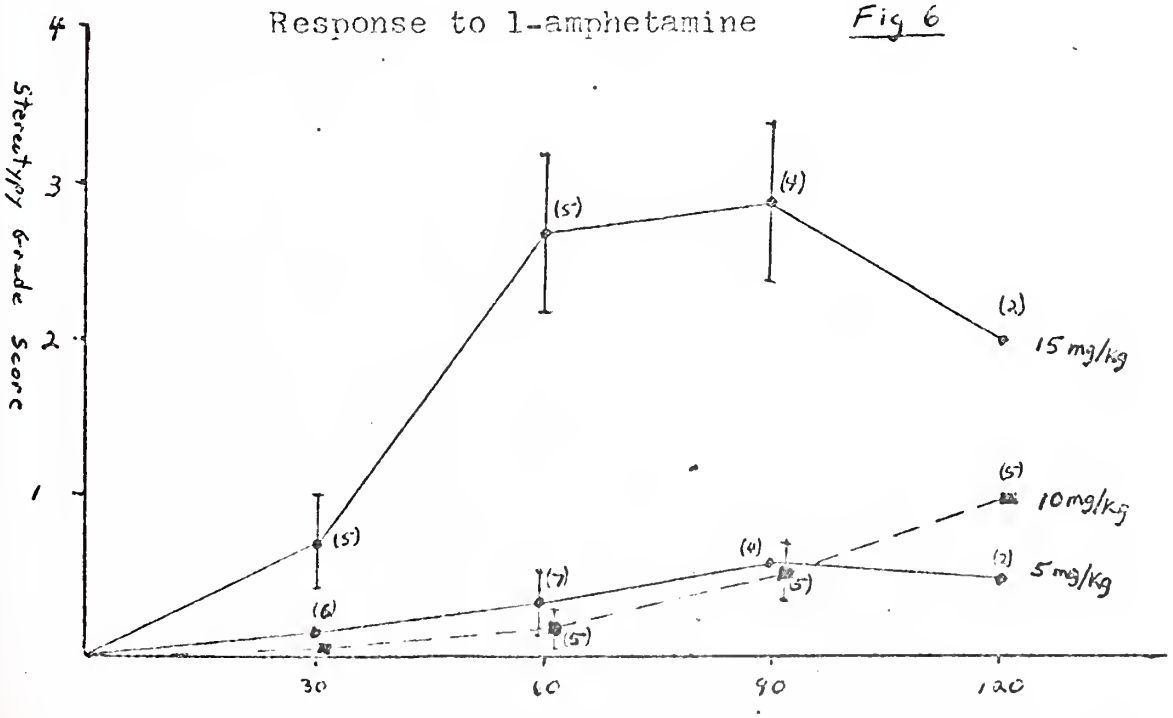
Results are shown in figures 5 and 6.

The results of this experiment agree temporally with previous reports, stereotypy becoming maximal in the second hr. post injection. Taylor and Snyder (1970) however reported an ED_{50} for AISB to the l isomer of 8.8 mg/kg. In this test only one out of five animals showed any significant stereotypy at 10 mg/kg of l-amphetamine, and even at 15 mg/kg of the l isomer the intensity of oral activity (licking and gnawing) was not as marked as that after 5 mg/kg of the d isomer.

AISB to d and l isomers of amphetamine in varying doses after prior administration of pargyline 100 mg/kg, 2 hrs earlier



number in parenthesis represents number of animals scored at that time
each point represents the average stereotypy grade score of all animals scored at that time ± S.E.M.



Of particular note is that all animals administered l-amphetamine at 5 and 10 mg/kg exhibited a peculiar periodic staring behavior in which the animals would remain immobile, with eyes widely dilated and proptotic but not responding to visual threat. This behavior was also marked by some at least moderate degree of catalepsy as determined by the animal leaving a rear leg propped up by a cork for 1 to 2 min. The cataleptic nature of the behavior was difficult to determine however due to the periodic nature of the appearance of the behavior for 30 sec. to 2 to 3 min. at a time interspersed with the animal's more normal stereotypyed or other activities. It was further noted that in those animals spontaneously demonstrating this behavior, the behavior could be induced and intensified by presenting the animal an auditory stimulus (hand clapping etc.), immediately after the cessation of which this behavior would be prominently displayed. This behavior was not observed with the higher dose of 15 mg/kg of l-amphetamine and not in any of the animals administered the d isomer in this experiment.

The usefulness of MAO inhibitors for investigations into the mechanism of action of amphetamine in AISB might however be questioned. Such pretreatment alters the function of all CNS monoamine systems, not just DA, and may thus become a confounding factor in attempts to determine the role any one particular system in this behavior. To avoid this possible problem it was decided to use amphetamine without prior MAO

inhibition in all the remaining experiments.

2) d and l amphetamine without prior MAO inhibition

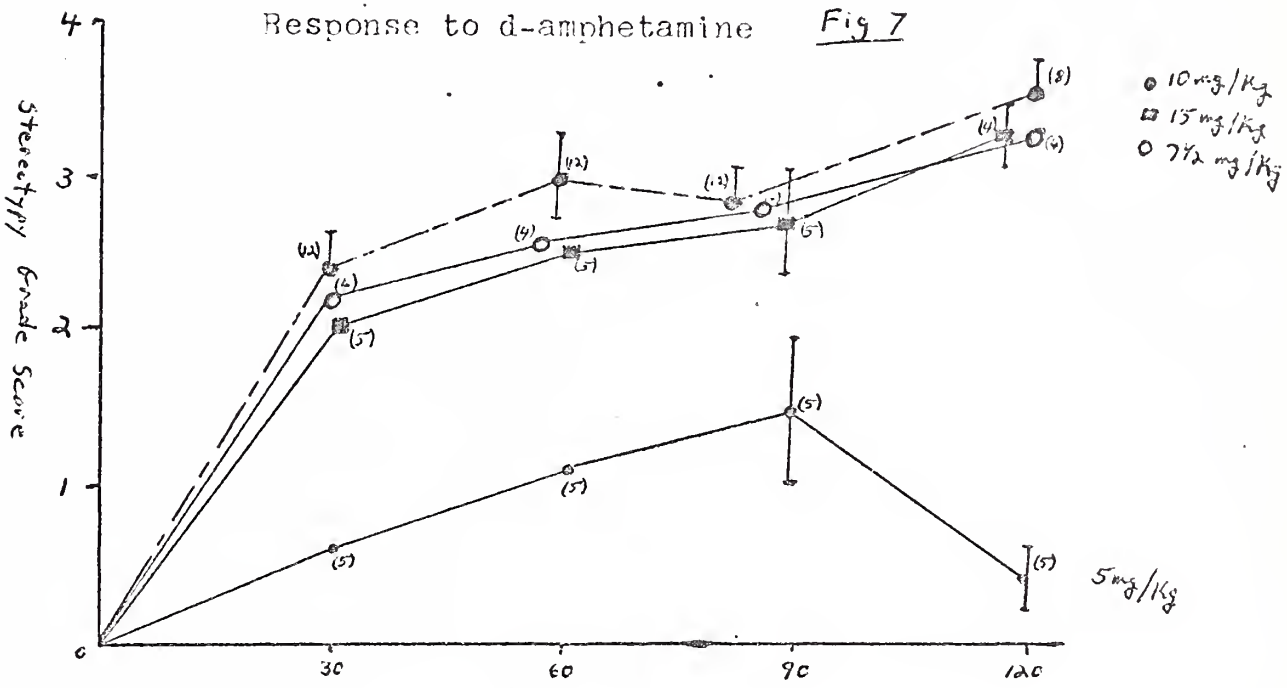
The results of administering varying doses of d (5, 7.5, 10 and 15 mg/kg) and L (10, 20, 25, and 30 mg/kg) amphetamine to rats without pargyline pretreatment are seen in figures 7 and 8.

Comparision of figures 5 and 6 with figures 7 and 8 show that pargyline did indeed bring on stereotypy faster and stronger than in nonpretreated animals. Pargyline pretreated animals given 5 mg/kg of d-amphetamine showed a maximum stereotypy of 3.5 at 60 min. post injection, while nonpretreated animals with this dose showed a maximum stereotypy of only 1.5 at 90 min. It might also be noted that the low doses of l-amphetamine did not alter the normal social interaction of the animals in a grouped situation (huddling), which was blocked by all doses of d-amphetamine.

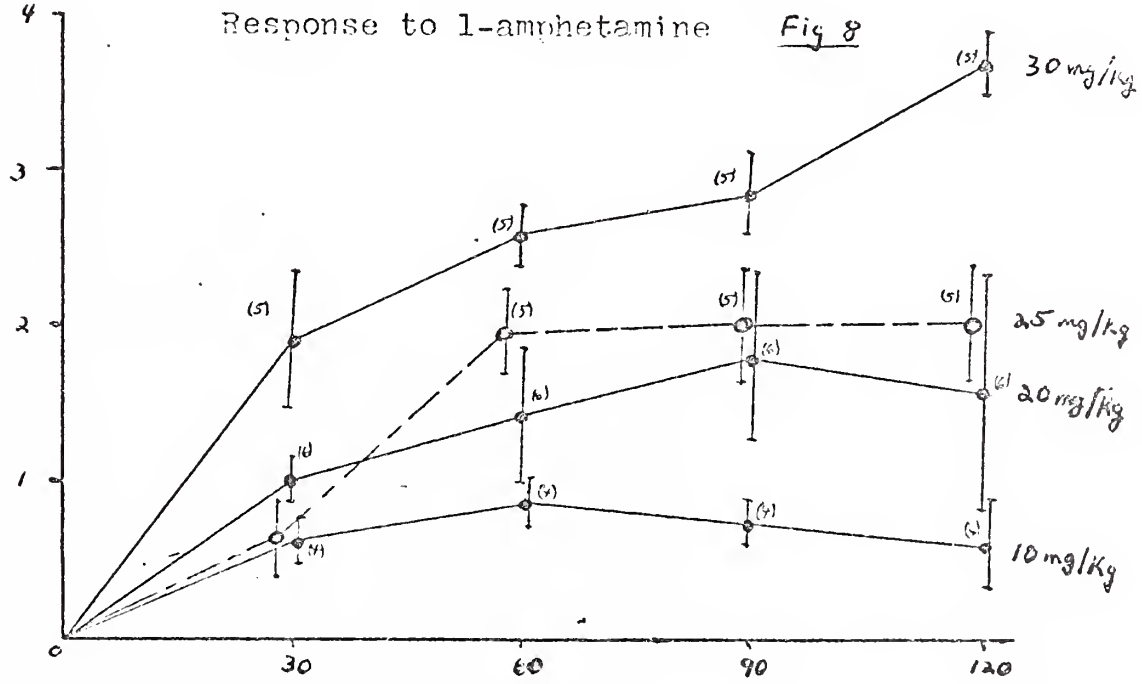
Figures 9 and 10 show dose response relationships of AISB to the d and l isomers for the nonpretreated animals. The results show a minimum ration of 3:1 in the efficacy of d:l in inducing stereotypy. The greatest change in responsiveness to d occured between 5 and 7.5 mg/kg and for l between 25 and 30 mg/kg (figure 7). The ED_{50} for production of grade 3 stereotypy or above was between 5 and 7.5 mg/kg for the d isomer and approximately 25 mg/kg for the l isomer (figure 8). A similar ratio of 3-4:1 seems also to hold from this data for the efficacy of d:l to elicit grade 2 stereotypy or better. These results disagree

AISB to d and l isomers of amphetamine in varying doses, without previous MAO inhibition

Response to d-amphetamine Fig 7



Response to l-amphetamine Fig 8



Dose Response of AISB to d and l isomers of Amphetamine

Fig 9

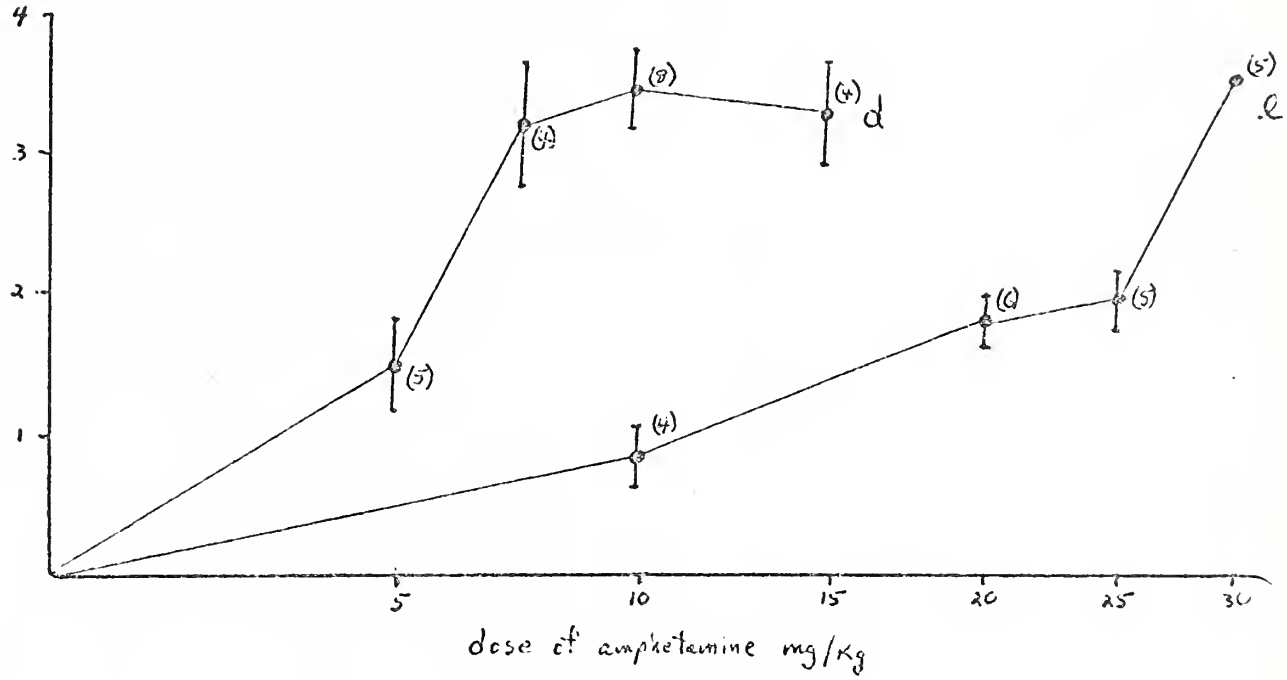
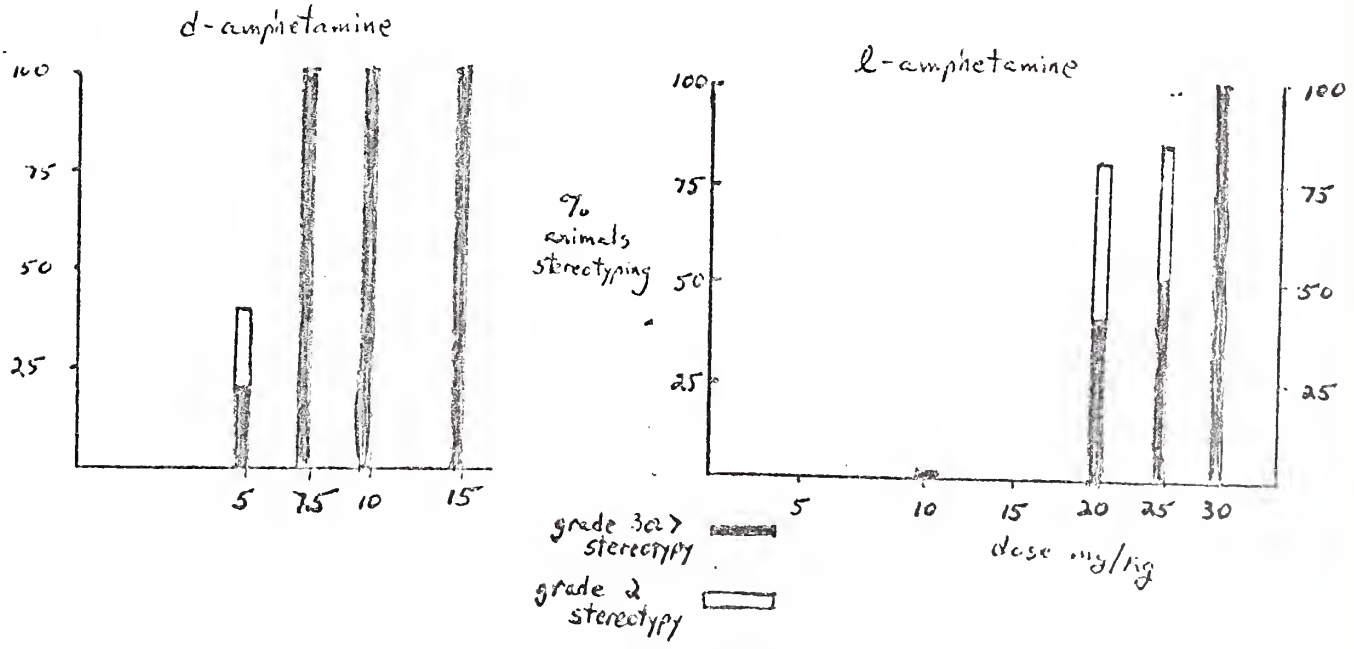


Fig 10



with the 2:1 ration found by Taylor and Snyder (1971) but do agree with the 3-4:1 difference in potencies of d:l amphetamine in stimulating release from DA synapses in vivo in the rat as reported by Von Voigtlander and Moore (1973). Further examination of this finding appears in the final discussion.

3) d-amphetamine with phenoxybenzamine pertreatment

It has been well demonstrated that amphetamine can induce stereotyped behavior in the absence of CNS NE. It has not however been noted whether such stereotypy is changed temporally or qualitatively from that seen in normal animals.

Del Rio and Fuentes (1972) reported that concommitant with their reversal of stereotypy, neuroleptics also produce a return of locomotor stimulation after amphetamine. This finding may bear directly on the problem of the latency of onset of AISB as opposed to that induced by apomorphine. Taylor and Snyder (1970) have suggested that AISB is predominantly DA mediated whereas locomotor stimulation seems to be predominantly NE mediated. It would be possible for the initial hyperactivity of animals after amphetamine to represent a period of NE dominance, replaced after a period of about one hr. by a switch to a state of relative DA dominance manifested by the onset of stereotypy. Thus by administering neuroleptics that would block DA receptors, Del Rio and Fuentes were able to return the animals to an NE dominant state and observed the return of locomotor stimulation which would normally have been replaced by stereotypy at the time. It would seem reasonable from this hypothesis to expect

that if NE activity were blocked, and amphetamine thus led to DA stimulation alone, the stereotypy observed might be more similar to that seen with apomorphine.

To test this hypothesis 5 rats were pretreated with phenoxybenzamine, an α-adrenergic blocking agent (Anden et al., 1967), 20 mg/kg i.p., 20 min. before testing of the animals with d-amphetamine 10 mg/kg.

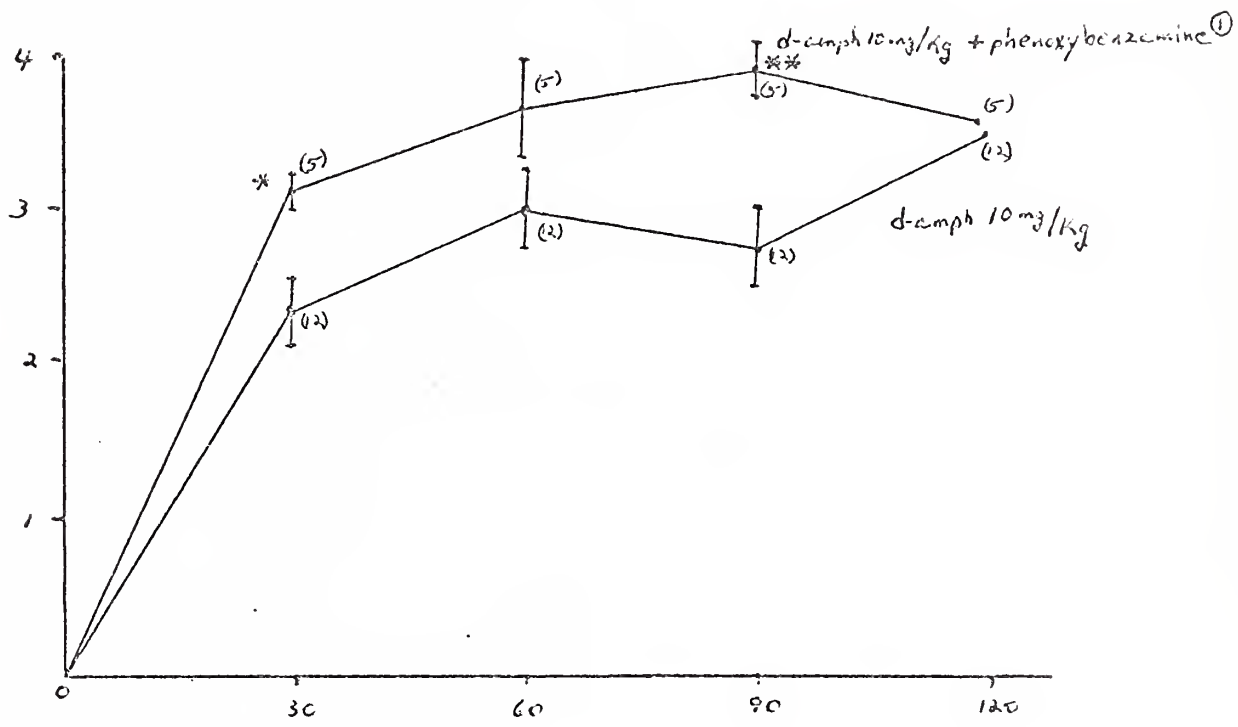
Results are seen in figure 11.

The average stereotypy grade score for the phenoxybenzamine pretreated animals was significantly greater ($p < .05$, Student's t test) than that of the nonpretreated animals at 30 min. Furthermore, the intensity of the gnawing behavior in the pretreated group was considerably greater than that seen in animals administered amphetamine alone. (At 90 min. this difference was significant at level of p .01) Thus, this preliminary experiment was consistent with the hypothesis that the delay in onset of AISB is due to an initial NE predominance.

In a further test of this hypothesis rats were administered clonidine, a potent NE receptor agonist (Anden et al., 1970), at a dose of 3 mg/kg. Three rats received the clonidine 30 min. prior to injection of d-amphetamine 10 mg/kg and three more rats received the clonidine 30 min. after injection of the amphetamine. Pretreatment with clonidine did not slow the onset of stereotypy nor did it diminish stereotypy when administered to animals after amphetamine. In fact, all the six animals after having received both clonidine and amphetamine demonstrated the most intense gnawing behavior witnessed by this investigator. It might be

Fig 11

Comparison of AISB to d-amphetamine 10 mg/kg with and without phenoxybenzamine pretreatment



1) phenoxybenzamine 20 mg/kg administered 20 min. before amphetamine

* Differs from nonpretreated animals at level of $p < .05$ (students t-test)

** Differs from nonpretreated animals at level of $p < .01$ (students t-test)

noted also that those animals administered clonidine after amphetamine exhibited after clonidine injection extreme hyperactivity - often jumping right out of the cage. This hyperactivity lasted for approximately 40 min. and was accompanied by intense gnawing activity.

Naylor and Olley (1972) have reported that lesions of the nuc. lateralis amygdaloideus, which receives NE but not DA innervation, led to a reduction of gnawing behavior elicited in rats by apomorphine. The above results with clonidine would agree with a stimulatory role for NE in gnawing behavior, but do not readily agree with the hypothesis relating delayed onset of AISB to initial NE dominance. The actions of clonidine seem to be multiple however. While it is a direct NE receptor stimulant in the mg/kg dosage range, it also appears to be most potent (ug/kg) in stopping the firing of NE cells in the locus coeruleus by a direct effect on these cells. (Aghajanian, personal communication). It is thus difficult to draw any firm conclusions from the above findings. It does seem however clear that though NE may not be necessary for amphetamine to induce stereotyped behavior, altering the level of NE activity can effect the quality of the stereotypy observed.

4) Role of 5-HT in AISB

Though no experiments were performed in this study to specifically elucidate the role of serotonin in AISB it seems worthwhile at this point to mention some of the work done on this problem.

Ernst (1972) has reported that pretreatment of rats with

parachlorophenylalanine, a specific serotonin depleting agent (Koe and Weissman, 1966), did not alter the stereotyped behavior elicited by d-amphetamine (1 mg/kg) and MAO inhibition. Thus, he concluded that the DA effect to produce AISB was not mediated through 5-HT. McKenzie (1973) however, has reported some preliminary findings in which electrolytic lesions of the mid-brain raphe nuclei, site of the 5-HT containing cell bodies (Dahlstrom and Fuxe, 1965), blocked the appearance of apomorphine induced stereotyped gnawing, without altering licking or sniffing. Cools (1973) has found that injection or 10ug 5-HT into the antero-ventral caput caudati of the cat produced intense choreoathetosis and self-directed activities. 5-hydroxytryptophan, the immediate precursor of 5-HT, is capable of eliciting in MAOI pretreated rats a fierce compulsive gnawing syndrome (Ernst, 1973 and personal observation). This behavior is unlike that induced by amphetamine in that the animal holds his teeth clamped over the mesh wire for prolonged periods of time while performing choreoathetoid movements of his front paws. This behavior is directly attributable to serotonin since it is not altered by prior depletion of catecholamines by AMPT (Ernst, 1972). Thus, though 5-HT is not necessary for AISB it seems possible that alteration of central 5-HT activity may modify the type of stereotypy observed. Evidence has also accumulated to suggest that central 5-HT may exert an inhibitory influence on the locomotor stimulatory effect of amphetamine (Mabry and Campbell, 1973). If 5-HT should prove antagonistic to NE in

the inducement of locomotor activation, this might provide yet another means by which 5-HT might alter stereotypy. It should also be noted that the doses of amphetamine (> 5 mg/kg) required to induce SB are capable of depleting the extra granular stores of 5-HT in the terminals of serotonergic neurons (Fuxe and Ungerstedt, 1970).

5) Stereotypy to low doses of apomorphine

It was of further interest to examine the nature of the behavioral response to apomorphine in low doses. It is known that apomorphine induced stereotypy has a log-dose relationship (Lal and Sourkes, 1973), but the qualitative nature of the response to sub and nearthreshold doses has not been described.

Apomorphine HCL was administered to 3 animals each, in each of three doses levels - 1, 2 and 3 mg/kg. The 3 animals receiving 1 mg/kg showed sniffing, elevation of their heads and periodic immobile staring behavior. At 2 mg/kg there was a greater diversity of effects. 1 animal showed level 2 stereotypy with eyes closed and occasional gnawing motions of its mouth: 1 animal showed sniffing of the air with head elevated, rearing against the cage wall and periodic staring behavior; the last animal alternated equally between grade 3 stereotypy and periodic staring behavior. At 3 mg/kg all animals showed the normal stereotypyed compulsive gnawing snydrome with marked ptosis.

It was surprising to find that subthreshold doses of apomorphine elicited the same periodic staring behavior seen with low doses of l amphetamine after pargyline pretreatment. It was

possible to induce reduced levels of SB to apomorphine by the administration of very low doses.

Result of Lesions

Initial attempts to obtain specificity in lesions of the ML or NS pathways by intracerebral injection of 6-OHDA into the mid-brain were unsuccessful. Due to the closeness of the structures and diffusion of the injected solution both pathways were always effected. This led to the attempt to lesion the terminal areas of the two systems where the spatial separation of structures would not make diffusion of the injection so critical. Injections of 6-OHDA into the head of the caudate, the olfactory tubercle and the mesolimbic pathway were performed bilaterally as described in methods.

Examination of the histoflourescent brain sections of operated animals revealed:

1) Necrosis around the injection site of the 6-OHDA at the cannula tip encompassed an area of about 0.3 mm^2 in its greatest extent. This area showed marked generalized tissue necrosis and a large amount of orange autoflourescence.
2) Catecholamine flourescence about this necrotic area was greatly reduced to varying extents depending upon the precise location of the injection. In general an area of at least 2.0 mm^2 immediately around the injection site would be totally devoid of catecholamine flourescence. Surrounding area would be partially depleted. The specificity of the 6-OHDA for catecholamines was evidenced by the fact that in areas totally depleted of green catecholamine flourescence the yellow specks of 5-HT terminals were

still observed.

3) When the cannula tip was in the region of a fiber bundle the injected 6-OHDA had a tendency to diffuse along the length of the fiber bundle. Thus, the anterior commisure and the "pencil bundles" of Wilson were often marked by orange autoflourescent material for considerable lengths after injections into the tubercle and caudate respectively.

Thus, the intracerebral injection of a large amount of 6-OHDA (40ug) produced a surprisingly small degree of generalized necrosis and was capable of depleting large areas of DA terminal regions. Due to the normal great intensity of green flourescence in the olfactory tubercle, nuc. accumbens and caudate-putamen, the borders of depleted areas, though they gradually shaded off, were always readily discernable.

Table 1 shows the results of 8 animals lesioned bilaterally in the olfactory tubercle.

6 of the 8 animals showed significant loss of flourescence in the tubercle and accumbens. Only 3 animals (89-04, 89-02, 96-10) however, showed a reduced level of stereotypy when tested with d-amphetamine 10 mg/kg. These 3 animals all showed significant loss of striatal flourescence. 96-10, with less than half the striatal flourescence present on one side and only about 10% on the other side did not stereotype at all with amphetamine. When tested with apomorphine 5 mg/kg 96-10 and 89-02 showed the normal stereotyped compulsive gnawing. Animals 86-01, 89-01 and 89-05 showed great depletion of

Table 1

Histochemical and Behavioral Effects of

6-OHDA Lesions of Forebrain Limbic DA Areas

animal	Stereotypy to d-amph[1]	apo[2]	% Intactness[5] O.T.[3]	N.Ac.[4]	Caudate[8] (L)	(R)
86-01	4[6]		40	10	100	100
86-04	4[6]		60	80	100	100
86-05	4[6]		70	70	100	100
89-01[10]	3 stare[7]		5	10	99	99
89-05	2-3 stare		0	0	80	70
89-04[10]	2 stare		0	0	70	50
89-02	1-2 stare	4	2	0	60	75
96-10[9]	0 stare	4	5	5	40	10

1) Stereotypy grade score to d-amphetamine 10 mg/kg

2) Stereotypy grade score to apomorphine 5 mg/kg

3) O.T. - olfactory tubercle, 4) N.Ac - nuc. accumbens

5) % Intactness = % flourescence of strucüure as compared to that of a nonlesioned animal (see Methods)

6) 86-01, 86-04, and 86-05 were tested with d-amphetamine 5 mg/kg 2 hr after pretreatment with pfagyline 100 mg/kg

7) Periodic. immobile, staring behavior as described in text

8) Caudate nuc. , left and right, from most rostral pole to level of 7190 (Konig and Klippel)

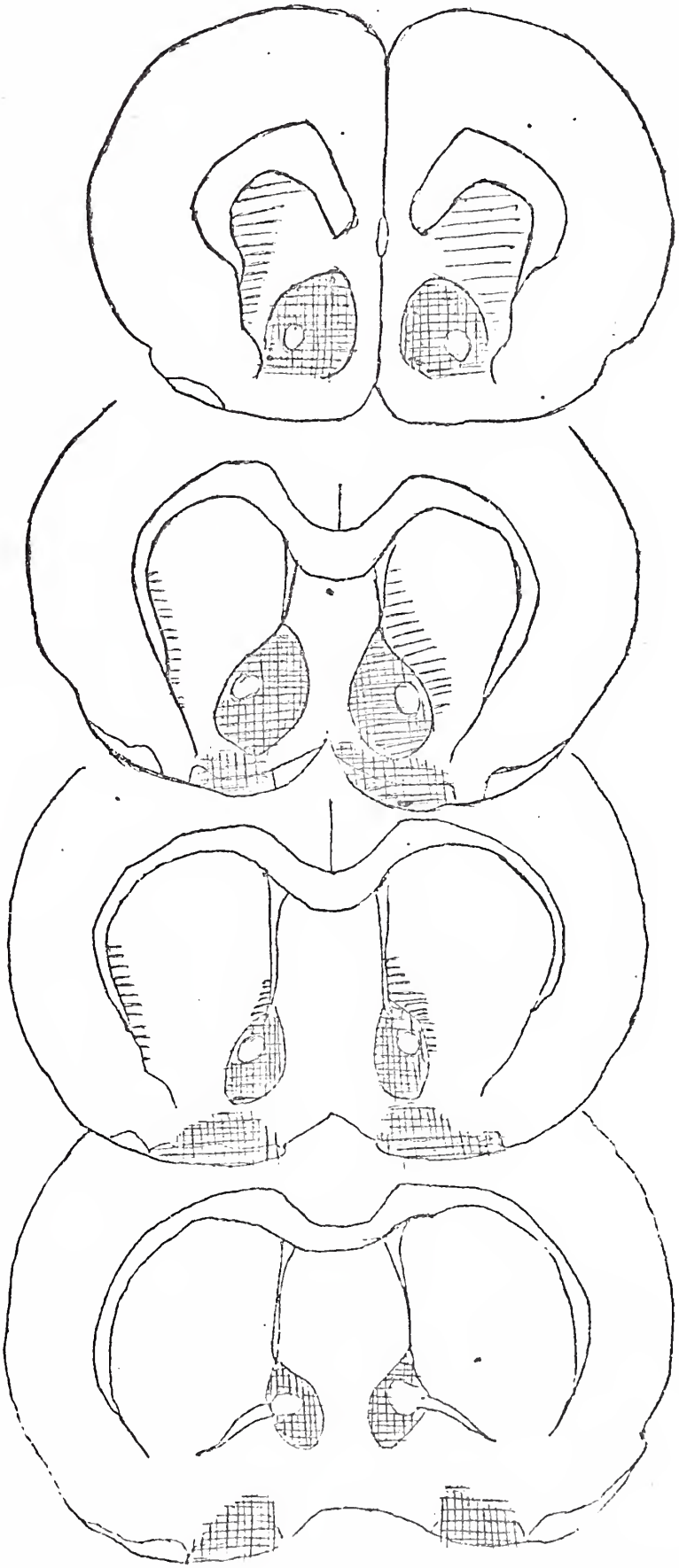
9) 96-10 lesioned with 20ug 6-OHDA in the ML pathway at level of 7470 bilaterally; all other animals shown here lesioned with 40 ug 6-OHDA bilaterally into the olfactory tubercle

10) For graphic representation of histoflourescence see figs 12+13

animal-89-01

Histoflourescence after 6-OHDA Lesion of O.T.

Fig 11.



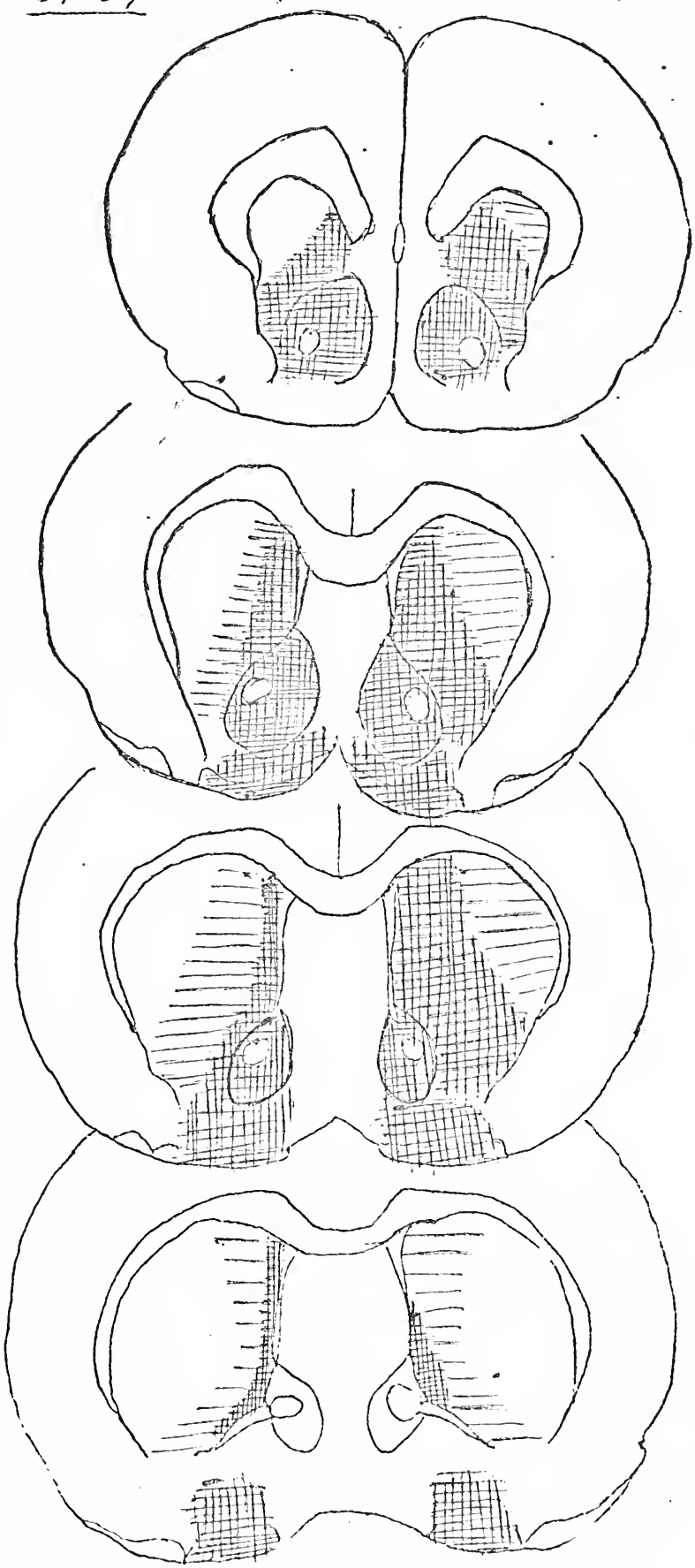
Total depletion of native catecholamine flourescence

Partial depletion of flourescence

animal - 89-04

Histoflourescence after 6-OHDA Lesion of O.T.

Fig 13



flourescence from tubercle and accumbens while striatal flourescence was intact. These animals all stereotyped normally to amphetamine.

Animal 89-01 is the most clearcut of the group. This animal showed essentially total loss of flourescence in accumbens and tubercle with only the most minimal striatal involvement, (see figure 12). This animal showed a normal high grade of stereotypy to amphetamine. It would appear then that DA in the olfactory tubercle and nuc. accumbens is not necessary for amphetamine to elicit stereotyped behavior. Graphic representations of the flourescence depletion of animals 89-01 and 89-0 as representatives of this group are shown in figures 12 and 13.

It is of note that all those animals that did show great depletion of limbic DA demonstrated a peculiar staring behavior when administered amphetamine. This behavior was periodic in appearance and seemed identical to that seen with low doses of l-amphetamine administered to normal rats after pargyline pretreatment. The rats were immobile with eyes markedly proptotic, not responding to visual threat. The behavior could be stimulated by auditory stimulation as described earlier. This behavior appeared for 1 to 2 minutes at a time interspersed with the animal's more normal stereotyped activities.

Thus, depletion of DA from tubercle and accubens resulted in the appearance of a peculiar periodic staring behavior but did not prevent AISB. The dorsal diffusion of 6-OHDA iniected into the tubercle resulted in the depletion of varying amounts

of striatal dopamine in several animals. Those animals with significant striatal depletion showed reduced levels of stereotypy. Animal 96-10 with the greatest amount of striatal depletion did not stereotype to amphetamine but did to apomorphine. These results tended to indicate that it was striatal dopamine that was necessary for AISB.

To test this hypothesis a second series of experiments was performed in which injections were made directly into the caudate nucleus as described in methods.

Table 2 shows the results of bilateral injects of 6-OHDA into the caput caudati.

Animals 96-05 and 96-07 showed total loss of flourescence in the striatum while the limbic areas were involved to varying extents. These animals did not stereotype to amphetamine. 96-08 which was found to have 50% depletion of striatal flourescence bilaterally and totally intact limbic flourescence did not stereotype to d-amphetamine. 96-09 which showed an intermediate level of caudate depletion also showed an intermediate level of stereotypy, varying between grade 1 and grade 3 throughout the time observed. 96-06 in which the caudate was not depleted of flourescence to any large extent showed a normal stereotyped response to d-amphetamine. Graphic representation of histoflourescence of animals 96-05 and 96-08 as representatives of this group is shown in figures 14 and 15.

Thus, it was possible by depleting 50% of the striatal DA, without any involvement whatever of the limbic areas, to completely abolish AISB. Animals 96-07, 96-08, and 96-05

Table 2

Histochemical and Behavioral Effects of

Bilateral 6-OHDA Lesions of Anterior Caudate Nucleus

Animal	Stereotypy To d-amph	Stereotypy To apo	% Intactness OT	% Intactness NAc.	% Intactness Caudate (L)	% Intactness Caudate (R)
96-05 (1)	EA	4!	60	60	0	0
96-07	EA stare	3-4	40	50	0	0
96-08 (1)	EA	4	100	100	50	50
96-06	3-4		90	90	90	50
96-09	1-3		100	100	40	90

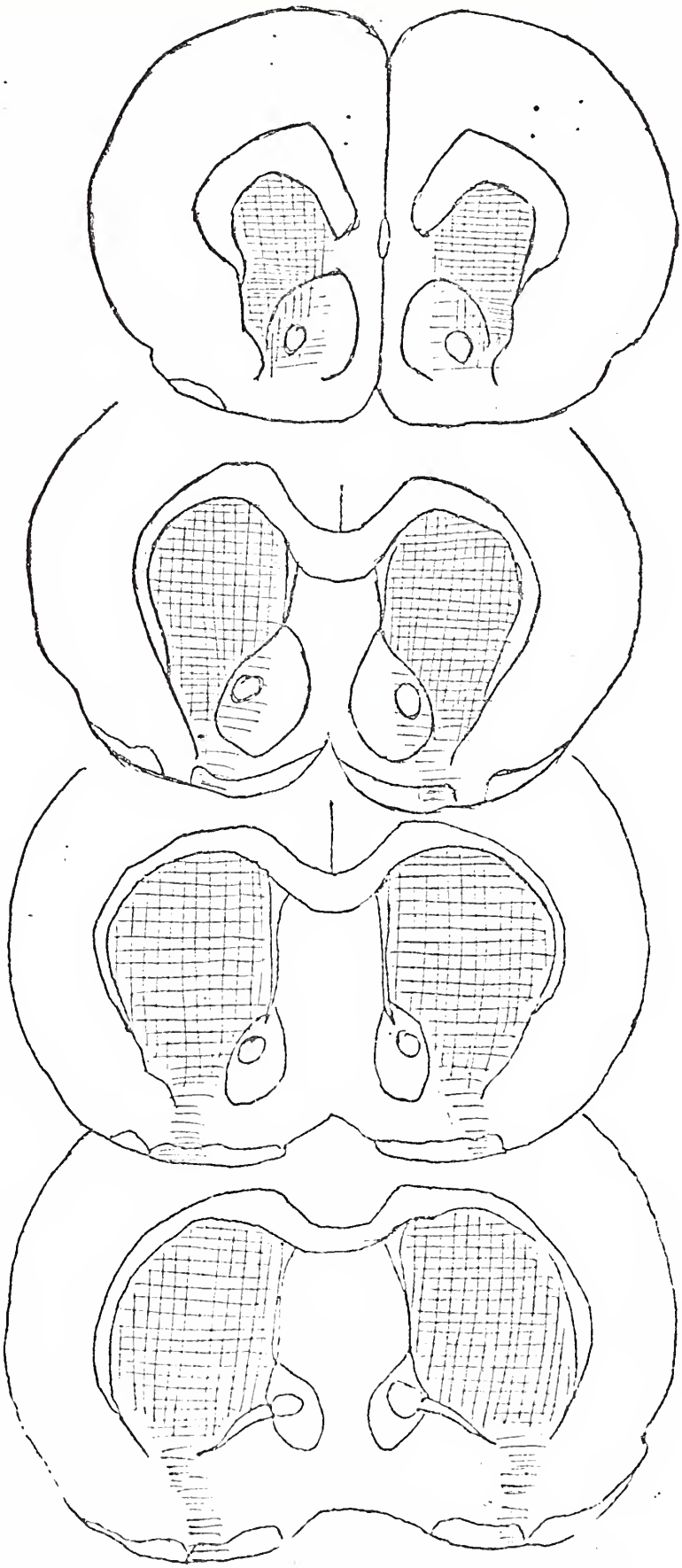
Animals shown here were lesioned with 40 ug 6-OHDA bilaterally into the head of the caudate nucleus as described in methods

1) For graphic representation of flourescence see figs 14 + 15

animal
96-05

Histoflourescence after 6-OHDA Lesion of Corpus Striatum

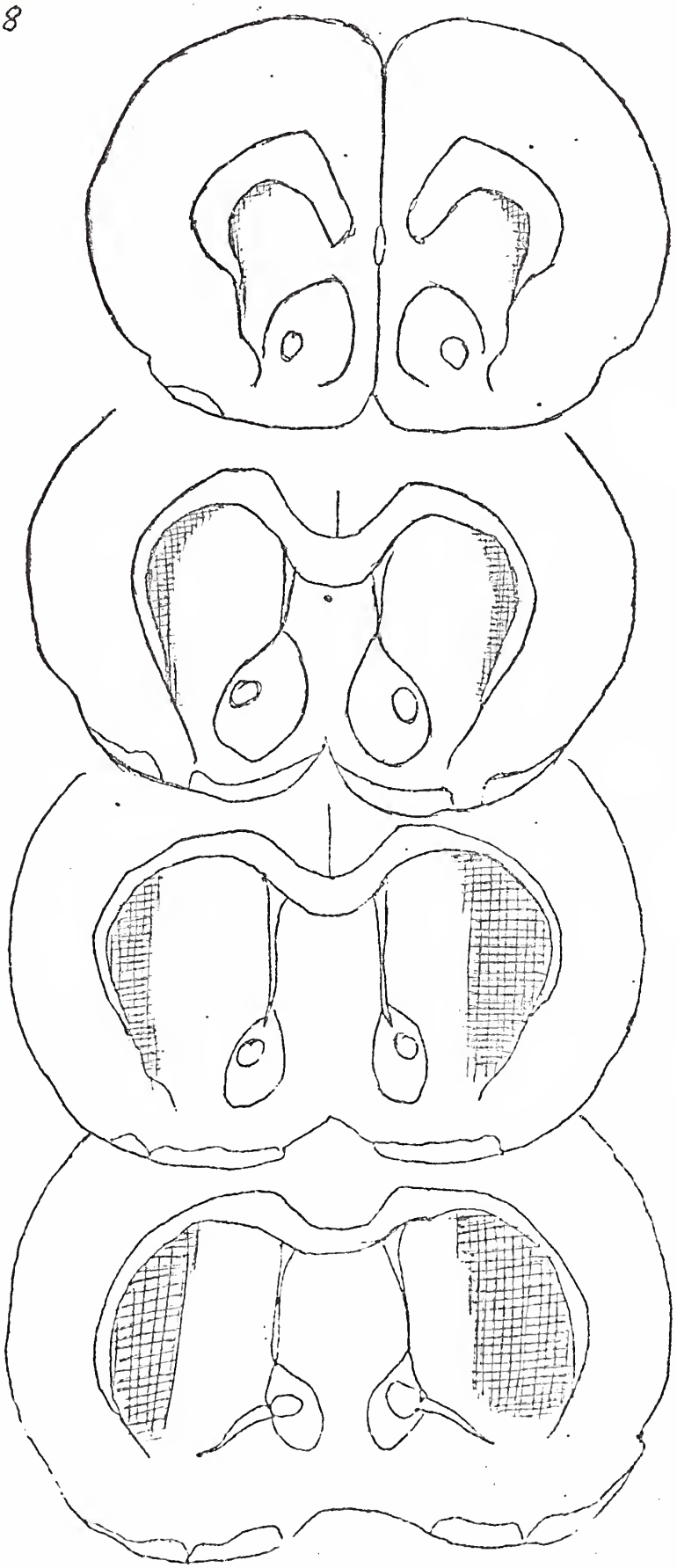
Fig 1



animal
96-08

Histoflourescence after 6-OHDA Lesion of Corpus Striatum

Fig 15



all stereotyped normally to apomorphine proving that the post-synaptic receptors were intact. 96-05 showed a particularly intense gnawing behavior to apomorphine, perhaps due to a denervation hypersensitivity of the striatal DA receptors. 96-07, the only animal in this series with significant limbic flourescence depletion again demonstrated the previously described periodic staring behavior to amphetamine.

It is of note that those animals with significant striatal DA depletion and subsequent loss of stereotypy to d-amphetamine ambulated around their cage throughout the time of amphetamine effect. Though no quantitation of the locomotion was made, it was clearly evident that these animals continued to show a locomotor response to the drug when normal animals were stereotyping. Furthermore, it is of note that these animals continued to show marked sniffing activity.

It should be noted that in tubercle lesioned animals there was always some degree of loss of cortical NE terminals. This was due to the involvement of the median forebrain bundle which traverses the limbic dopamine areas dividing accumbens from tubercle. In most cases this involvement was minimal however. The nuc. striaterminalis dorsalis and ventralis were variously effected in some of the tubercle lesioned animals, especially when diffusion of 6-OHDA along the anterior commisure was evident. Involvement of these structures did not seem to correlated in any consistent manner however, with the behavioral changes described. This was also true for involvement of the nuc. septi medialis and lateralis. With caudate lesions it was possible to

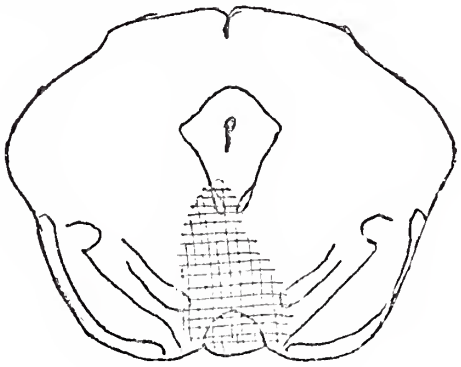
totally spare involvement of all limbic structures. The amygdala, being caudal to the caudalmost extent of brain sectioned was not examined. It is unlikely however, that this structure would have been involved.

Many of these animals lost weight after lesioning. Those animals that lost the most weight were the animals lesioned in the caudate. These animals were clearly adipsic and aphagic for about one week after lesioning and had to be tube fed to keep them through this period. They eventually began eating again by about ten days but stayed at about 60% their initial prelesion weight. The caudate lesioned animals were also extremely placid and manageable showing a fair degree of catalepsy by not moving when placed on a tilted mesh surface. The tubercle lesioned animals were not noticeably different in their behavior from nonlesioned animals before drug testing.

Some animals had initially been lesioned in the midbrain. Figure 16 shows the histology after one such lesion (electrolytic, 8 mAmps x 60 sec.). The area of involvement includes the ascending ML and NS tracts bilaterally. This animal fell to about 60% of its prelesioned weight. When tested with d-amphetamine 5 mg/kg and pargyline 10 days after lesioning it failed to stereotype and showed the periodic staring behavior. This animal also showed intermittent autofellatory activities to amphetamine plus pargyline, which were also noted in some animals with small electrolytic lesions of the tractus striohypothalamicus administered amphetamine plus pargyline.

Fig. 16

Result of electrolytic lesion of ventral midbrain
8 m Amps x 60 sec



A 1760

hatched area shows necrotic tissue

Thus, it was possible by depleting striatal dopamine to abolish AISB, and the loss of AISB appeared dependent only on the volume of striatal DA depleted. Both antero-medial and antero-lateral lesions could decrease or block AISB (compare figures 13 and 15). Limbic DA does not appear to be necessary for this effect of amphetamine. Furthermore, it was demonstrated that the loss of stereotypy was due solely to the loss of striatal dopamine since apomorphine was still able to elicit stereotyped behavior in these animals.

Discussion

These experiments have demonstrated that it is the nigro-striatal dopamine tract that mediates amphetamine induced stereotyped behavior. Dopamine in the olfactory tubercle and nuc. accumbens does not appear to be in any way necessary for amphetamine to induce this behavior. These conclusions have been reached by lesioning the forebrain terminal areas of these two pathways with 6-OHDA. It was possible to completely abolish AISB by the depletion of DA from the striatum, without any involvement of the limbic areas. On the other hand, lesion of the nuc. accumbens and olfactory tubercle without striatal depletion of DA did not prevent AISB but did result in a periodic staring behavior upon drug testing. Furthermore, it was demonstrated that the striatal lesions prevented stereotypy solely by the depletion of presynaptic DA stores, since the administration of apomorphine did produce the normal stereotyped response in these animals.

Though never definitively proven, it has been thought that stereotypy to stimulant drugs was mediated by striatum ever since Amsler prevented apomorphine stereotypy by lesioning this area (Amsler, 1923). Three recent papers, however, made it appear very likely that AISB might have been mediated through the meso-limbic pathway. Simpson and Iversen (1971) reported that bilateral lesions of the substantia nigra in rats did not alter AISB. The histology presented by these authors however makes it apparent that the entire extent of nigra was not

lesioned. In the present experiments it was found that depletion of a considerable degree of the striatal DA bilaterally was necessary to abolish AISB. Thus, the negative finding might be attributable to incomplete lesions. These authors also used a scheme of quantitation of head movements for scoring of stereotypy. These experiments have shown that lateral head movements, which appear in grade 2 stereotypy, are not an adequate measure of stereotypy by themselves. Graduation to grade 3 and 4 stereotypies in the present experiments was determined by the onset of compulsive oral activities. Thus, the nigral lesioned animals reported by Simpson may in fact have exhibited a lower grade of stereotypy than controls that the authors were unable to see due to their scoring scheme. The second paper by Iversen on this subject (Creese and Iversen, 1972) reported the success of 6-OHDA lesions of the NS pathway to prevent AISB. The coordinates given by the authors for their cannula placement made it obvious however that they had lesioned <u>both</u> the NS and ML pathways. Since their only control for efficacy of lesion was the determination of striatal tyrosine hydroxylase content they did not in fact have any proof for the specificity of their lesion. The present experiments have justified the conclusion of AISB mediation by the NS system, which was unjustly inferred from the previous work. It is of note that in this second paper Iversen reported that the locomotor stimulant effect of the "nigral" lesioned animals, as determined by photocell counts/min. was lower in the second hour after injection of

amphetamine than was the control group. This loss of locomotor stimulation is usually attributed in the second hr. post amphetamine to the onset of stereotypy. It is likely that the decrease in photocell counts/min. in the second hr. post amphetamine in Iversen's "nigral animals" was due to the appearance of the periodic, immobile staring behavior which would have resulted from the additional lesion of the mesolimbic DA pathways in these animals. These authors did note that the depletion of striatal dopamine did not prevent the locomotor stimulation to amphetamine, as was also observed in the present experiments.

McKenzie (1972) reported that suctioning out the olfactory tubercle significantly decreased or abolished stereotypy to apomorphine. He postulated from this that limbic dopamine mediated the stereotyped response. He does note however that destruction of neural tissue other than dopaminergic may have been responsible for the inhibition of stereotyped behavior. The present experiments confirm this possibility. It may be noted that electrolytic lesions of extensive (but not complete) portions of NAc and NAc-OT performed by this investigator, resulted in the modification of stereotyped response in some animals, but did not prevent that stereotyped response to amphetamine. It is more problematic however, to explain McKenzie's report in the same paper that lesions of the striatum did not significantly reduce stereotyped behavior to apomorphine. The present experiments and all other previous reports on the relation of striatum to stereotypy have shown that striatal lesions reduce

stereotypy to drugs (Amsler, 1923; Fuxe and Ungerstedt, 1970; Anden, 1970; Naylor and Olley, 1972).

Thus, the present experiments confirm the hypothesis that AISB is in fact mediated through the nigro-striatal system. The loss of stereotypy seems to parallel the extent of striatal depletion. Fuxe (Fuxe and Ungerstedt, 1970) reported that small electrolytic lesions in the dorsal anterior striatum seemed capable of preventing the biting aspect of AISB, and suggested from this that a topographic organization of stereotyped responses might exist in the striatum. The present experiments show the loss of stereotypy to be proportional to the extent of anterior caudate depleted, apparantly independent of the specific area involved. As long as 50% of the anterior caudate is depleted of dopamine, be it the vento-lateral 1/2 or the dorsomedial 1/2, the animal will not stereotype. The graded responses of animals to lower doses of both amphetamine and apomorphine also tend to link degree of stereotypy to the degree of striatal DA stimulation.

The findings reported here of decreased body weight after 6-OHDA lesions agree with Ungerstedt (1971), who has implicated interruption of the NS pathway in the MFB as the crucial factor in lateral hypothalamic lesions to account for the classic behavioral findings of adipsia and aphagia. Those animals in the present experiments that showed the most marked adipsia and aphagia, requiring tube feeding for a week after the lesion, were the animals lesioned in the striatum.

The caudate has classically been conceived of as a

structure involved in the control of movement. The well known links of motor cortex-caudate-globus pallidus - VA and VL nuc. of the thalamus - motor cortex, and the many motor disturbances resulting from striatal pathology have all strengthened this concept. Recent physiologic and behavioral studies have suggested however, that the caudate may also be involved in the regulation of complex behavioral activities (for review of this literature see Cools, 1973 and Brodal, 1969). Kemp and Powell (1971) have concluded from detailed light and electron microscopic examination of the synaptic organization of the caudate that this structure must elaborate a highly integrated and modified version of the messages received by it from cortex. It is of note that all cortical areas project to caudate (Kemp and Powell, 1970). While there is somatotopic organization of input, any one area of striatum will be played upon by two cortical regions and receive numerous input from adjoining areas of striatum. Furthermore, it is of note that the medial portions of the head of the caudate are projected to by orbital frontal cortex, insula and opercula and in return project to the internal segment of the globus pallidus which projects to the intralaminar thalamic nuclei (esp. CM) and ventral midbrain tegmentum as well as to the VL nuc. of the thalamus. Thus, this medial portion of caudate becomes a link betwen limbic and motor systems. Kemp and Powell note (1970)

> If this correlation is correct, it is tempting to speculate that its significance lies in the recent evidence for a final convergence of the three major sensory pathways, through association cortical connections, in the depths of the superior temporal sulcus, at the frontal pole and in the cortex of the frontal operculum, and that all these regions are interconnected with each other and with the cortex on the orbital surface of the frontal lobe and with the temporal pole. It is possible, therefore, that through these regions of cortex the medial parts of the striatum and the internal segment of the globus pallidus are receiving an influence which represents a complex integrating not only of all the sensory pathways but also of the areas of "association" cortex of the frontal and parietotemporal lobes.

Such a juncture of sensory, associative and motor information processing in the medial striatum would be a most attractive place for a lesion to account for schizophrenia. Perhaps it is an alteration of dopamine in the medial striatum that lies at the basis of this disorder. Thus, the stereotypies of thought so characteristic of this disorder may result from an aberrant processing of associative cortical material in the medial striatum just as the motor stereotypies result from an aberrant processing of motor impulses. Both Klawans (1972) and Cools (1973) have suggested the striatum as the site of pathogenisis for schizophrenic psychosis.

One of the major factors mitigating against the striatum as seat of pathogen sis in psychosis would seem to be that of the effect of anticholinergic medications. As previously discussed, while such medications successfully alleviate the extrapyramidal side effects of the neuroleptics they do not seem to block the antipsychotic effects of these drugs.

In this regard it is interesting to reexamine clozapine. As previously mentioned, this drug demonstrates good antipsychotic potency but does not produce extrapyramidal side effects. It also does not block apomorphine induced stereotypy. Furthermore, it increases the turnover of DA in the limbic areas to a greater extent than it does in the striatum. It was hypothesized that clozapine might represent a case of DA receptor specificity differentiating between NS and ML systems. Another possibility however is that the anticholinergic effects of clozapine tend to autoalleviate any of the extrapyramidal symptons it might induce, and tend to potentiate the stereotypy that the drug would attempt to block. If this were the case, then receptor differentiation between ML and NS systems need not be invoked. Snyder (unpublished) has recently offered evidence along these lines, showing that clozapine had the greatest anticholinergic potency of the neuroleptics tested in his lab, and, furthermore, that the ability of these medications to induce extrapyramidal effects seemed to be inversely proportional to their anticholinergic potency.

Thus, alteration of cholinergic tone can effect stereotyped behavior and extrapyramidal motor symptoms, both shown to be striatal phenomena, but apparently not psychosis. Perhaps then psychosis is mediated through the mesolimbic pathway.

The incidence of psychosis in Parkinsonian patients treated with L-Dopa is greatest in that segment of the population with organic brain syndromes, and this psychosis is accompanied 70%

of the time by oral dyskinesias (Celesia and Barr, 1970). Iversen (1971) has reported that lesions of frontal cortex potentiate stereotypy in the rat. It may be hypothesized that frontal cortex exerts some sort of inhibitory influence on DA mediated activity in the striatum. Fuxe and Ungerstedt (1970) have reported that transections separating frontal pole and olfactory bulb from the rest of the brain, very similar to the transection used clinically for frontal leucotomies, were capable of potentiating the behavioral effects of amphetamine in rats. In these rats, clonidine administration was capable of eliciting behavioral activation, exploratory activity and biting, which resulted in unlesioned animals only after the administration of apomorphine along with clonidine. It has also been reported that the incidence of tardive dyskinesias is extremely high in frontal leucotomized patients receiving neuroleptics. The neuroanatomic link of frontal motor cortex with striatum is classically known. The link of associative, orbital frontal cortex with medial striatum has been discussed. It should also be noted that frontal orbital cortex is probably linked to olfactory tubercle also. Thus, those patients with organic brain syndromes who manifest evidence of frontal lobe disease seem to show an increased sensitivity to both the motor and psychic effects of L-Dopa therapy. It might also be wondered to what extent the frontal degeneration in patients with Huntington's chorea adds to their hypersensistivity to striatal DA. It would be of considerable

interest to know the mechanism of this frontal cortical influence on dopamine activity. It does not appear to be the result of direct alteration of dopamine levels (Iversen, 1971). Is it mediated through acetylcholine? GABA? If psychosis were mediated by the ML pathway and motor phenomena by the NS it might be of interest to know if tumors or gun-shot wounds etc. of non-orbital frontal cortex, since no projection from this area to tubercle and accubens is known, would result in an increased sensitivity to L-Dopa induced dyskinesias without accompanying psychosis.

Thus, though it has been established that AISB is mediated by striatal dopamine, it is still not at all clear whether AISB is in fact a valid animal model for psychosis.

The initial drug studies reported here resulted in several interesting findings. The threshold doses for stereotypy to the d and l isomers were considerably greater than those previously repoted by Snyder (Taylor and Snyder 1970). (5.0-7.5 mg/kg for d and approx. 25 mg/kg for l as opposed to Snyder's report of 2.1 for d and 4.4 for l) Part of this difference is accounted for by Snyder's pretreatment of his animals with an MAO inhibitor, but even with pargyline pre-treatment in these studies the threshold response to l amphetamine was greater than 10 mg/kg. (It is interesting to note that in a recent report by Lal and Sourkes (1973) the ED_{50} for stereotypy to d-amphetamine was determined to be 2.8 mg/kg. These investigators compared their result very favorably

to that of the 2.1 mg/kg reported by Snyder. Snyder however pretreated his animals with an MAO inhibitor, Lal and Sourkes did not. It is thus very likely that two different criteria for stereotypy were used). More importantly however, the ratio of effective dose was found to be 3-4:1 (l:d) to elicit AISB. This was clearly greater than the 2:1 reported by Snyder. The present finding agrees very nicely,however, with the report of VonVoigtlander and Moore (1973) of a 3-4 x greater potency of the d isomer than the l to stimulate release of DA in vivo in the rat. The present result also tends to agree more with Bunney and Aghajanian (1973) who find that the d isomer is 6-10 times more powerful than the l in producing a 50% inhibition of spontaneous firing rate of DA neurons. This data all mitigates against the proposed equipotency of d and l amphetamine on DA neurons as inferred by Snyder from his data. In this regard the report by Angrist (1972) of the equipotency of the d and l isomers of amphetamine in eliciting amphetamine psychosis becomes problematic to a DA theory of psychosis. This was a very small study however, and needs to be repeated. Such a finding may indicate a role for NE in the induction of psychosis. In this regard it should be noted that the antipsychotic neuroleptics,in addition to their DA receptor blockade and anticholinergic properties, also possess α-adrenergic blocking abilities. The significance of this action for their clinical effectiveness is unknown.

It has been suggested (Fuxe and Ungerstedt, 1970)

that the particular type of behavior elicited by amphetamine, locomotor stimulation or stereotypy, is dependent upon the relative activities of the NE and DA systems. If NE activity is dominant one sees locomotor activation, if DA prevails then stereotypy emerges. Since amphetamine is more potent on NE release than DA release (Azzuro and Rudledge, 1973), the lower threshold for LS than SB agrees with this hypothesis. The finding of a speedier onset and stronger degree of stereotypy to d-amphetamine after pretreatment with phenoxybenzamine also supports this hypothesis. The results of treatment with phenoxybenzamine and clonidine further indicate that while NE many not be necessary for the induction of AISB, alteration of NE tone can alter the nature of the stereotypy observed, though not in any simple manner.

In early time periods after d-amphetamine when NE predominance may be expected, the behavior observed is LS. If NE is blocked, then SB becomes manifest earlier. If additional NE stimulation is added to an animal who already has a high level of DA activity however, the increased NE increases the intensity of the SB. Thus, clonidine led to intense gnawing when administered to d-amphetamine treated animals. But for a period of approximately 30-40 min., those animals given clonidine 30 min. after amphetamine also displayed extreme LS behavior (violent jumps) intermittently with their gnawing. At these high levels of stimulation of both DA and NE systems, the

alternation between LS! and SB! may result from small momentary imbalances of activity of the 2 amine systems. The animals administered clonidine prior to amphetamine did not show a delay in onset of SB. Thus, it might be that levels of SB are dependent on the attainment of certain absolute levels of DA stimulation. While decreasing the NE tone may hasten the onset of SB, once the requisite DA activity has been achieved, increasing NE will only intensify the SB.

It must also be noted that doses of amphetamine sufficient to induce stereotypy (greater than 5mg/kg) are capable of stimulating release of the extragranular store of 5-HT (Fuxe and Ungerstedt, 1970). Though the role of 5-HT in AISB is not clear, as previously discussed, there does seem to be a 5-HT inhibitory influence on the locomotor stimulation of amphetamine (Mabry and Campbell, 1973). The autofellatory behavior seen with d-amphetamine and pargyline treatment of rats lesioned in the ventral tegmentum (involving the ascending DA pathways) or in the tractus striohypothalamicus (of the substantia innominata, whose relation to " ventral striatum " has been discussed) may involve an abberant interaction of DA and 5-HT systems resulting from the lesions. This abnormal sexual activity was not seen in rats that received amphetamine without pargyline. 5-HT has been noted to play a role in the control of sexual activity. Angrist (personal comm.) has noted that pretreatment of dogs with parachlorophenylalanine prevents the increased sexual activity normally seen after amphetamine administration.

Thus, it is probable that the activities of all the monoamine systems as well as that of the cholinergic system will all combine to effect the stereotypy observed. In investigating the effect of any particular drug on AISB it would thus be desirable to know its actions on all of these systems and not merely on the DA system alone.

Grade 1 stereotypy, which consisted of sniffing the air with occaisional head movements, was seen in many animals prior to the onset of higher levels of stereotypy and was especially prominent in those rats administered low doses of l-amphetamine. Azzaro and Rutledge (1973) have demonstrated that d-amphetamine shows a decreasing potency of release of amines from brain slices in the order NE > DA > 5-HT with NE being the amine released at lowest concentrations of amphetamine and 5-HT release requiring the highest concentration. Bunney (pers. comm.) has found that the l isomer of amphetamine is more potent in inhibiting the firing of cells in the locus coeruleus (an NE cell group) than in the A9 cell group, suggesting the l isomer to be a potent NE releaser (unlike Snyder's earlier report of a low potency of the l isomer on the NE system). Thus, with low doses of l-amphetamine, which tend to stimulate the NE system more than the DA, and in the early stages of the drug effect with d-amphetamine, when the low drug levels would probably result in a greater NE than DA effect, we find the animal's head up sniffing the air. (This grade of stereotypy was also observed in rats 1 mg/kg of apomorphine. However, since the behavior after apomorphine is almost immediate in onset and of very short duration with low doses, it would not be unexpected for the animal to have an increased NE tone during

this period due to the excitement of the injection). Amphetamine is known to alter postural reflexes. With increasing degrees of DA activity in later time periods after d-amphetamine the animal progresses from this predominant extensor state to a predominant flexor state, exhibiting the typical hunched posture. Since sniffing was seen even in the caudate lesioned animals that did not stereotype at all to amphetamine, it must be concluded that the sniffing induced by amphetamine is not mediated by the same striatal DA system that mediates SB, or perhaps not by striatal DA at all. Sniffing would not then seem to be a part of the stereotyped behavior induced by amphetamine, but rather a result of the generalized activation produced by the drug, probably mediated predominantly by NE.

Anden (Anden et al., 1973) and Svensson (1971) have offered considerable evidence to indicate that DA receptor stimulation as well as NE stimulation is necessary for the locomotor activation produced by amphetamine. It was noted in these experiments that in animals administered low doses of l-amphetamine, locomotor stimulation did not appear as marked as that seen with d-amphetamine. This may have resulted from the predominant NE stimulation without DA stimulation that may have resulted from the low doses of the l isomer. If correct, such an explanation would agree with the necessity for DA stimulation in LS. The observation of decreased LS was only casual however. Formal testing of the LS response to l-amphetamine would seem worthwhile. The ability of d-amphetamine or apomorphine +

clonidine to increase motility and reactivity of reserpine pretreated striatomized rats led Anden(1970) to postulate a role for DA receptors outside the striatum in the regulation of activity and alertness. Naylor and Olley(1972) have also reported that striatal lesions which did block stereotypy to amphetamine did not alter the locomotor response. The animals here, in which striatal dopamine was depleted, and who did not show AISB, did show a prolonged motor stimulation. Thus, these experiments agree with Anden and suggest further that limbic DA may be involved in the locomotor activation produced by amphetamine.

A peculiar periodic staring behavior was observed in animals administered low doses of l-amphetamine after MAO inhibition. It was of considerable surprise to once again see this behavior exhibited by animals depleted of limbic DA when they were tested with d-amphetamine. This behavior was seen in animals given d-amphetamine only if limbic DA was depleted. It is possible that the staring episodes seen with low doses of l-amphetamine + pargyline and in ML lesioned animals administered d-amphetamine may result from a predominant NE stimulation without the requisite limbic DA activation to allow for a normal active response. (For further discussion of this behavior and its possible significance refer to Appendix).

It has thus been demonstrated that loss of DA from OT and NAc does not prevent AISB, but does seem to alter the locomotor response to amphetamine. Observations of LS in this study were not however quantified. Such quantification of LS by use of an Annimex activity meter in NS and ML 6-OHDA lesioned animals

might serve to more precisely define the role of one or the other of the DA systems in LS induced by amphetamine.

It has been demonstrated that 6-OHDA induced lesions of anterior caudate do block AISB. This effect is due solely to the loss of striatal DA, since apomorphine can still induce normal stereotypies in these animals. The present study has limited the area of lesion to anterior caudate, rostral to A 7190. It would be of interest know if lesions of more caudal caudate are also effective in blocking AISB. While the present study has tended to indicate that the reduction of stereotypy is proportional to the amount of striatum depleted of DA, a somatotopic organization of stereotyped responses has not been ruled out. The present method of subjective approximation of of decreased flourescent intensity as a measure of DA depletion might be extended by spectroflourophotometric analysis to yield more reliable quantitation of DA loss.

While it has been established that AISB is mediated by the nigro-striatal DA pathway, the validity of AISB as an animal model of psychosis is still uncertain.

Appendix

Periodic Staring Behavior - possible relation to Catatonia

Naylor and Olley(1972) reported that concurrent with the decreased AISB resulting from lesions of the caudate was the appearance of a " periodic cataleptic behavior." Their first two grades of this behavior describe the animals as " alert appearance but no motor activity. Indifferent to observer and brief response to touch, inability to correct suspended position." This description sounds remarkably close to that of the periodic staring behavior described in these experiments. Naylor and Olley commented

> The finding of a cataleptic type behavior in animals with bilateral lesions of the caudate nucleus, caudate-putamen and globus pallidus after administration of amphetamine was surprising... no previous reference could be found suggesting any cataleptic ability(of amphetamine). A present understanding of the proposed mechanism of action of amphetamine does not suggest an explanation.

Since an apparantly identical behavior could be elicited to d-amphetamine in rats depleted of limbic DA, two possible explanations for Naylor and Olley's findings present themselves. First, the lesions may not have been specific for striatum and may have encroached upon OT and NAc. Unless this were specifically looked for it might easily be missed, especially if one's attention were directed to avoiding involvement of the internal capsule as was Naylor and Olley's. A second possibility is that the neural processing to effect the motor responses mediated by limbic DA involves the striatum. Hence, the gross destruction of striatal tissue might mimic the effect of specific limbic DA depletion.

(This once again points out the difficulty of drawing conclusions about the role of "striatal DA" as a result of altered behavioral response after lesions producing generalized striatal necrosis or loss).

Wilson(1972) in his examination of the neuroanatomy of the NAC quotes at length a description of behavior elicited in cats by stimulation of the accumbens.

> The second response consistently obtained was evoked by prolonged stimulation of the floor of the remaining portion of the frontal horn of the ventrical, between the head of the of the caudate and the septum, at or just in front of the anterior commisure. After a latent period of 1 to 5 sec, the pupils dilated moderately, following which respirations became much more rapid, the depth and rate increasing with the duration of stimulus. The animal showed no other signs which could be interpreted as pain, fear, or rage, but sat or stood rather tensely. When the stimulus had continued for 5 to 10 sec, there was a sudden burst of activity in the form of violent running and springing movements which continued for 1/2 min or more after the stimulation ceased and gradually subsided. At the end of the outburst the animal sat or stood on the table as quietly as before, the pulse and respirations rapidly returning to normal...
>
> (Rioch and Brenner,1938)

Elicitation of behavior by stimulation of brain regions must always be critically examined to rule out spread of exciting current to to other areas. Nonetheless, the description of tense, motionless cats with dilated pupils is very similar to the staring behavior described in these experiments with rats. Furthermore, the violent springing of the cats is quite comparable to a peculiar behavior observed in some rats by Fuxe and Ungerstedt(1970).

> After bilateral electrocoagulations of the neostriatum, without damage to other structures, interesting behavioral effects were observed following treatment with a high dose of amphetamine (5-10 mg/kg) or apomorphine(5 mg/kg) with clonidine(2 mg/kg). Marked hyperactivity with running around and stereotyped activity were no longer observed. Instead, the rats were now immobilized most of the time but performed very fast intermittent jumps, which were jerky in character and ended rapidly.

Fuxe and Ungerstedt concluded that " increased DA receptor activity in the limbic forebrain area may be of importance in this behavior."

Evidence thus seems to be accumulating to involve limbic DA areas in the control of levels of motor activity. It is interesting to speculate on what the appearance might be in humans of disorders in this area.

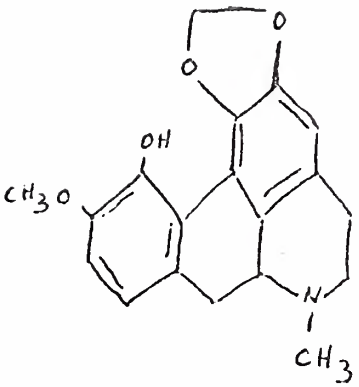
> Catatonic symptoms... are psychomotor acts frequently seen in schizophrenic patients and by no means limited to the so-called catatonic type. The two most extreme symptoms are catatonic stupor and catatonic excitement. In stuporous states, patients are mute and immobile, they are negativistic and will neither eat nor drink; they do not take care of their body needs... They seem to be less bothered by fatigue than normal persons would be... Those stuporous patients that can be induced to give accounts of their experience speak about a strange dream-like state... The striking thing about stupor is that the musculature is dissociated very much the way a patient can be "taught" in hypnosis that his will and his actions can be separated... Catatonic excitemnt is a severe state of agitation with sudden onset and indiscriminate violence.
>
> (Redlich and Freedman,1966)

> ... Even when the patient seems immobile and stuporous, he looks so tense and explosive that those around him feel apprehensive and uncomfortable - and not without reason for he may become excited and assaultive without obvious provocation.
>
> (Detre and Jarecki,1971)

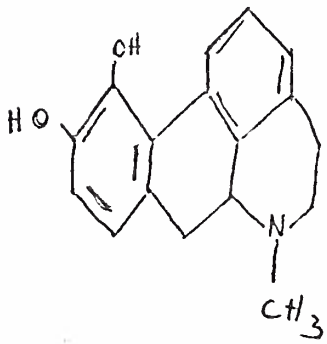
Indeed, it was only with the utmost reluctance that this investigator determined the cataleptic nature of the periodic staring behavior, because the appearant tenseness of the rat made him think the animal would spring violently at the slightest touch. Elkes(1957) has reported that some catatonics administered methamphetamine appeared to sleep - a most bizarre and paradoxical response to this normally alerting drug. Furthermore, bulbocapnine has long been used to elicit a catatonic like state in animals (DeJong,1945). The structur of bulbocapnine is remarkably similar to that of apomorphine. (See fig17). Bulbocapnine has also been reported to block AISB in rats (Tseng and Walaszek,1972).

It seems not unreasonable to suggest that a disorder of limbic dopamine may be involved in the pathogenesis of the peculiar disorders of motor behavior seen in catatonic schizophrenia.

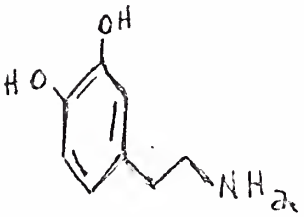
Fig 17



bulbocapnine



apomorphine



dopamine

Structural Similarities of Bulbocapnine, Apomorphine and Dopamine

Acknowledgements

I would like to express my sincere appreciation to all those who have helped in the conduction of these experiments and preparation of this manuscript. I should like especially to thank

Dr. George Aghajanian, who has been my guru in neuropsychopharmacology and whose enthusiasm, knowledge and humanity have been an inspiration

Dr. Steven Bunney, for innumerable helpfull discussions

Ms. Nancy Margiotta, for her patient, generous and expert help in the preparation of histologic materials

Ms. Elaine Shrewsbury, for her generous assistance with the care of sick animals and preparation of photographic materials

Dr. Terry Powley, for the secret to keeping alive rats that don't eat

Drs Henry Haigler, Judy Walters, Dick Bandler, Torgny Svensson, Mike Davis and all the various members of the CMHC research team, who were always willing to lend a hand or idea, and whose warmth and humor made working a pleasant experience

Russ, Diane and Paul, who tolerated the vagaries of my psyche

The Scottish Rite, for their generous financial support

All the rats that gave their lives

Bibliography

1. Aghajanian, G.K. and Asher, I.M.; Histochemical Fluorescence of Raphe Neurons: Selective Enhancement by Tryptophan, Science 172, 1159, 1971

2. Amsler ; Archiv fur Experimentelle Pathologie und Pharmakologie 97, 1, 1923

3. Anden, N.E., Carlsson, A., Dahlstrom, A., Fuxe, K., Hillarp, N.A. and Larsson, K.; Demonstration and mapping out of nigro-neostriatal dopamine neurons, Life Sci. 3, 523, 1964

4. Anden, N.E., Rubenson, A., Fuxe, K., and Hokfelt, T.; Evidence for dopamine receptor stimulation by apomorphine, J. Pharm. Pharmac. 19, 627, 1967

5. Anden, N.E., Corrodi, H., Fuxe, K. and Hokfelt, T.; Increased impulse flow in bulbo-spinal noradrenaline neurons by catecholamine receptor blocking agents, Eur. J. Pharm. 2, 59, 1967

6. Anden, N.E., Corrodi, H., Fuxe, K., Hokfelt, T., Ryder, C. and Svensson, T.; Evidence for a central noradrenaline receptor stimulation by clonodine, Life Sci. 9, 513, 1970

7. Anden, N.E.; Effects of amphetamine and some other drugs on central catecholamine mechanisms, p 447, Amphetamine and Related Compounds, ed. Costa and Garattini, Raven Press, N.Y., 1970

8. Anden, N.E. and Stock, G.S.; Effect of clozapine on the turnover of DA in the corpus striatum and limbic system, J. Pharm. and Pharmacol. 25, 346, 1973

9. Anden, N.E., Strombom, U. and Svensson, T.; DA and noradrenaline receptor stimulation: reversal of reserpine induced suppression of motor activity, Psychopharmacologia 29, 289, 1973

10. American College of Neuropsychopharmacology - Food and Drug Administration Task Force; Neurological Syndromes Associated with Antipsychotic drug use, NEJM 289, 20, 1973

11. Angrist, B.M., Shopsin, B., Gershon, S.; Comparative psychotomimetic effects of stereoisomers of amphetamine, Nature 234, 152, 1971

12. Ashcroft, G.W., Eccleston, D., Waddell, J.L.; Recognition of amphetamine addicts, Brit. Med. J. 1, 57, 1965

13. Ayhan, I.H., Randrup, A.; Role of brain noradrenaline in morphine induced stereotyped behavior, Pschopharmacol. 27, 203, 1972

14. Azzaro, A.J., Rutledge, C.O.; Selectivity of release of NE, DA and 5-HT by amphetamine in various regions of rat brain, Biochem. Pharmacol. 22, 2801, 1973

15. Bell, D.S.; The experimental reproduction of amphetamine psychosis Arch. Gen. Psych. 29, 35, 1973

16. Blackwood, W., McMenemey, W.H., Meyer, A., Norman, R.M., Russell, D.S.; Greenfield's Neurophathology, Williams and Wilkins, Balt., 1963

17. Bloom, F.E., Groppetti, A., Algeri, S., Revuelta, A. and Costa, E.; Lesions of central NE terminals with 6-OHDA-biochemistry and fine structure, Sci. 166, 1284, 1969

18. Bolt, J.M.; Huntington's chorea in the west of Scotland, Brit. J. Psychiat. 116, 259, 1970

19. Bowers, M.B. and Freedman, D.X.; "Psychedelic" experiences in acute psychosis, Arch. Gen. Psych. 15, 240, 1966

20. Brodal, A.; Neurological Anatomy, 2nd Ed, Oxford U. Press, N.Y., 1969

21. Brothers, C.R.D.; Huntington's chorea in Victoria and Tasmania, J. Neurol. Sci. 1, 405, 1964

22. Bunney, B.S. and Aghajanian, G.K.; L-DOPA, amphetamine and apomorphine - comparative effects on firing rate of rat DA neurons, Nature 1973

23. Bunney, B.S. and Aghajanian, G.K.; Electrophysiological effects of amphetamine on dopaminergic neurons, in Frontiers in Catecholamine Research, ed. S.H. Snyder and E. Usdin, Pergamon Press, in press, 1973

24. Bunney, B.S., Walters, J.R., Roth, R.H. and Aghajanian, G.K.; Dopaminergic neurons - effect of antipsychotic drugs and amphetamine on single cell activity, J. Pharm. Exp. Ther. 185, 560, 1973

25. Carlsson, A. and Linqvist, M.; Effects of chlorpromazine and hloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain, Acta Pharm. Tox. 20, 140, 1963

26. Carlsson, A.; Amphetamine and brain catecholamines, in Amphetamine and Related Compounds, ed. Costa and Garattini, Raven Press, N.Y., 1970

27. Celesia, G.G. and Barr, A.N.; Psychosis and other psychiatric manifestations of levodopa therapy, Arch. Neurol. 23, 1970

28. Chapman, J.; The early symptoms of schizophrenia, Brit. J. Psychiat. 112, 225, 1966

29. Connell, P.H.; Clinical manifestations and treatment of amphetamine type of dependence, JAMA 196, 718, 1966

30. Cools, A.R.; The caudate nucleus and neurochemical control of behavior - function of DA and 5-HT in the caput nuclei caudati of cats, doctoral dissertation, Univ. Nijmegen, Drukkerij Brakkenstein, Nijmegen, Netherlands 1973

31. Cotzias, G.C., Van Woert, M.H. and Schiffer, L.M.; Aromatic Amino acids and modification of parkinsonism, NEJM 276, 1967

32. Coyle, J.T. and Snyder S.H.; Sci. 166, 899, 1969

33. Crane, G.E.; Tardive dyskinesia in patients treated with major neuroleptics: a review of the literature, Amer. J. Psychiat. 124 Suppl, 40, 1968

34. Creese, I. and Iversen, S.D.; Amphetamine response in rat after DA neuron destruction, Nature 238, 247, 1972

35. Dahlstrom, A. and Fuxe, K.; Evidence for the existence of monoamine containing neurons in the CNS 1. Demonstration of monoamines in the cell bodies of brain stem neurons, Acta Physiol. Scand. 62 Suppl 232, 1, 1965

36. deJong, H.H.; Experimental Catatonia, Williams and Wilkins, Balt., 1945

37. Del Rio, J. and Fuentes, J.A.; Further studies on the antagonism of stereotyped behavior induced by amphetamine, Eur. J. Pharm. 8, 73, 1969

38. Detre, T.P. and Jarecki, H.G.; Modern Psychiatric Treatment, Lippincott, Phil., 1971

39. Elkes, J.; Effect of psychotomimetic drugs on animals and man, in H.A. Abramson ed, Neuropharmacology, Transactions of the 3rd Conf, N.Y., Josiah Macy Jr. Foundation, 1957

40. Ellinwood, E.H.; Amphetamine psychosis- description of the individuals and process, J. of Nerv. and Mental Dis. 144, 273, 1967

41. Ernst, A.M. and Smelnik, P.; Site of action of DA and apomorphine in compulsive gnawing behavior in rats, Experientia 22, 837, 1966

42. Ernst, A.M.; Psychopharmacologia 10, 316, 1967

43. Ernst, A.M.; Relationship of the central effect of DA on gnawing compulsion in rats and the release of serotonin, Archives internationales de Pharmacodyn. et de Ther. 199, 219, 1972

44. Falconer, M.A.; Temporal lobe resection for epilepsy and behavioral abnormalities, NEJM 289, 451, 1973

45. Fog, R.L., Randrup, A. and Pakkenberg, H.; Aminergic mechanisms in the corpus striatum and AISB, Psychopharmacol. 11, 179, 1967

46. Fog, R.L., Randrup, A. and Pakkenberg, A.; Neuroleptic action of quaternary chlorpromazine and related drugs injected into various brain areas in rats, Psychopharmacol. 12, 428, 1968

47. Fog, R.; Stereotyped and nonstereotyped behavior in rats induced by various stimulant drugs, Psychopharmacol. 14, 299, 1969

48. Fog, R. and Pakkenberg, H.; Combined nitoman-pimozide treatment of Huntington's chorea and other hyperkinetic syndromes, Acta Neurol. Scandinav. 46, 249, 1970

49. Freeman, J.M., Aron, A.M., Collard, G.E. and MacKay, M.; Emotional correlates of Sydenharn's Chorea, Pediatrics 35, 42, 1965

50. Friedman, E. and Gershon, S.; L-Dopa and imipramine; biochemical and behavioral interaction, Eur. J. Pharm. 18, 183, 1972

51. Fuxe, K. and Hokfelt, T.; Catecholamines in the hypothalamus and pituitary gland, in Advances in Neuroendocrinology, Ganong and Martini eds., Oxford, 1969

52. Fuxe, K. and Ungerstedt, U.; Histochemical, biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration, in Amphetamine and Related Compounds, Costa and Garattini eds., Raven Press, N.Y., 1970

53. Griffith, J.D., Cavanaugh, J., Held, J. and Oates, J.A.; D-amphetamine: evaluation of psychotomimetic properties in man, Arch. Gen. Psych. 26, 97, 1972

54. Harnack; Path. Pharmakol. 2, 255, 1874

55. Hornykiewicz, O.; Dopamine and brain function, Pharmacol. Rev. 18, 925, 1966

56. Huntington; Med. and Surg. Reported, 1872

57. Janowsky, D.S., El-Yousef, M.K., Davis, J.M. and Selkerke, H.J.; Cholinergic antagonism of methylphenidate induced stereotyped behavior, Psychopharmacol. 27, 295, 1972

58. Janssen, P.A.J., Nniemigeers, C.J.E. and Schellekens, K.H.L.; Is it possible to predict the clinical effects of neuroleptic drugs from animal data?, Arzneimittel-Forschung 15, 104, 1965

59. Kamberi, I.A., Schneider, H.P.G., McCann, S.M.; Action of DA to induce release of FSHRF from hypothalamic tissue in vitro, Endocrinology 86, 278, 1970

60. Kemp, J.M. and Powell, T.P.S.; The cotico-striate projection in the monkey, Brain 93, 525, 1970

61. Kemp, J.M. and Powell, T.P.S.; Connexions of the striatum and globus pallidus-synthesis and speculation, Phil. Trans. R. Soc. Lond. B 262, 441, 1971

62. Klawans, H.L., Goetz, B.A., Westheimer, M.A.; Pathophysiology of schizophrenia and the striatum, Dis. of the Nerv. System 33, 711, 1972

63. Klawans, H.L. Rubovits, R., Patel, B.C. and Weiner, W.J.; Cholinergic and anticholinergic influences on AISB, J. Neurol. Sci. 17, 303, 1972

64. Klawans, H.L., Paulson, G.W., Ringel, S.P. and Barbeau, A.; Use of L-Dopa in the detection of presymptomatic Huntingtons chorea, NEJM 286, 1332, 1972

65. Koe, B.K. and Weissman, A.; JPET 154, 499, 1966

66. Kline, D.F. and Davis, J.M.; Diagnosis and Drug Treatment of Psychiatric Disorders, Williams & Wilkins, Balt., 1969

67. Konig, J.F.R. and Klippel, R.A.; The Rat Brain - A stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem, Krieger, N.Y., 1970

68. Kramer, J.C., Fischman, V.S. and Littlefield, D.C.; Amphetamine abuse, JAMA 201, 305, 1967

69. Lal, S. and Sourkes, T.L.; Potentiation and inhibition of the amphetamine stereotype in rats by neuroleptics and other agents, Archiv. internat. de Pharm. et de Ther. 199, 289, 1972

70. Lal, S. and Sourkes, T.L.; Ontogeny of stereotyped behavior induced by apomorphine and amphetamine in the rat, Archiv. internat. de Pharm. et de Ther. 202, 171, 1973

71. Mabry, P.D. and Campbell, B.A.; Serotonergic inhibition of catecholamine induced behavioral arousal, Brain Res. 49, 381, 1973

72. Mattson, R.H. and Calverly, J.R.; D-amphetamine sulfate induced dyskinesias, JAMA 204, 108, 1968

73. McKenzie, G.M.; Role of the tuberculum olfactorium in stereotyped behavior induced by apomorphine in the rat, Psychopharmacol. 23, 212, 1972

74. McKenzie, G.M.; Apmorphine induced aggression: characteristics, pharmacological intervention and site of action, Schizophrenia Bul., 1973

75. Nauta, H.J.H.; Hippocampal projection and related neural pathways to the midbrain in the cat, Brain 81, 319, 1958

76. Naylor, R.J. and Olley, J.E.; Modification of the behavioral changes induced by amphetamine in the rat by lesions in the caudate nuc., caudate-putamen and globus pallidus, Neuropharmacol. 11, 91, 1972

77. Nyback, H. and Sedvall, G.; Effect of chlorpromazine on accumulation and disappearance of catecholamines formed from tyrosine C 14 in brain, JPET 162, 294, 1968

78. O'Keefe, R., Sharman, D.F., Vogt, M.; Effect of drugs used in psychosis on cerebral DA metabolism, Brit. J. Pharm. 38, 287, 1970

79. Perry, T., Hansen, S. and Kloster, M.; Huntington's Chorea, Deficiency of GABA in Brain, NEJM 288, 337, 1973

80. Randrup, A. and Munkvad, I.; Stereotyped activity produced by amphetamine in several animal species and man, Psychopharmacol. 1, 30, 1967

81. Randrup, A. and Munkvad, I.; Biochemical, anatomical and psychological investigations of stereotyped behavior induced by amphetamine in Amphetamine and Related Compounds, Costa and Garattini eds., Raven Press, N.Y., 1970

82. Randrup, A. and Munkvad, I.; Evidence indicating an association between schizophrenia and dopaminergic hyperactivity in the brain, Orthomolecular Psychiat. 1, 2, 1972

83. Randrup, A. and Munkvad, I.; Role of catecholamines in the amphetamine excitatory response, Nature 211, 520

84. Redlich, F.C. and Freedman, D.X.; The Theory and Practice of Psychiatry, Basic Books, N.Y., 1966

85. Rioch, D. McK and Brenner, C.; Experiments on the corpus striatum and rhinencephalon, J. Comp. Neurol. 68, 491, 1938

86. Rylander, G.; Addiction to preludin i.v. injected, in Proc. of the 4th World Congress of Psychiatry, Madrid, Excerpta Medica Foundation, Amsterdam, 1966

87. Scheel-Kruger, J. and Randrup, A.; Stereotyped hyperactive behavior produced by DA in the absence of noradrenaline, Life Sci. 6, 1389, 1967

88. Sher, J.; Patterns and profiles of addiction and drug abuse, Arch. Gen. Psychiat. 15, 539, 1966

89. Simpson, B.A. and Iverson, S.D.; Effects of substantia nigra lesions on locomotor and stereotype responses to amphetamine, Nature, 230, 30, 1971

90. Singh, M.M., Smith, J.M.; Reversal of some therapeutic effects of an antipsychotic agent by an antiparkinsonian drug, J. Nerv. and Mental Dis. 157, 50, 1973

91. Slater, E. et al.; The schizophrenia-like psychosis of epilepsy, Brit. J. Psychiat. 109, p 95, 113, 130, 134, 143, 1963

92. Snyder, S.H.; Catecholamines in the brain as mediators of amphetamine psychosis, Arch. Gen. Psych. 27, 169, 1972

93. Stevens, J.; An anatomy of schizophrenia?, Arch. Gen. Psych. 29, 177, 1973

94. Stille, G. and Hippius, H.; Pharmakopsychiat., Neuropsychopharmakol. 4, 182, 1971

95. Svensson, T.H.; On the role of central catecholamines in motor activity, thesis for the dept. of pharmacology, Univ. of Goteberg, 1971

96. Taylor, K.M. and Snyder, S.H.; Amphetamine: differentiation by d and l isomers of behavior involving brain NE and DA, Sci. 168, 1487, 1970

97. Tseng, L.F. and Walaszek, E.J.; Blockade of d-amphetamine induced stereotyped behavior by bulbocapnine, Pharmacol. 7, 255, 1972

98. Ungerstedt, U.; 6-OHDA induced degeneration of central monoamine neurons, Eur. J. of Pharmacol. 5, 107, 1968

99. Ungerstedt, U.; Stereotaxic Mapping of the Monoamine pathways in the rat brain, Acta Phys. Scand., Suppl 367, 1971

100. Ungerstedt, U.; Adipsia and aphagia after 6-OHDA induced degeneration of the nigrostriatal DA system, Acta Phys. Scand. suppl 367, 1971

101. Yaryerra-Tobias et al.; Dis. N. System 31, 1970

102. Weissman, A., Koe, B., Tenen, S.; Anti amphetamine effects following inhibition of tyrosine hydroxylase, JPET 151, 339, 1966

103. Wilson, R.D.; Neural associations of the nuc. accumbens septi in the albino rat, thesis for M.S., dept. of psychology, M.I.T., 1972

104. Von Voigtlander, P.F. and Moore, K.E.; Involvement of the nigro-striatal neurons in the in vivo release of DA by amphetamine, amantadibe and tyramine, JPET 184, 542, 1973

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE
Dr. E. Mayeska Hosp of St Raphael New Haven, Ct	6/12/78
Phillip M. Gendelman YSM	12/6/78
Jill Greenwald - Biochem Dept. Yale College	12/14/79
M.F. Taylor YSM	